



## 510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY

### I Background Information:

#### A 510(k) Number

K213362

#### B Applicant

BioFire Defense, LLC

#### C Proprietary and Established Names

BioFire Global Fever Special Pathogens Panel; BIOFIRE SHIELD Control Kit for the BioFire Global Fever Special Pathogens Panel

#### D Regulatory Information

| Product Code(s) | Classification | Regulation Section                                                                                         | Panel             |
|-----------------|----------------|------------------------------------------------------------------------------------------------------------|-------------------|
| QVR             | Class II       | 21 CFR 866.4000 - Device To Detect And Identify Biothreat Microbial Agents In Human Clinical Specimens.    | MI - Microbiology |
| QMV             | Class II       | 21 CFR 866.3966 - Device to detect and identify selected microbial agents that cause acute febrile illness | MI - Microbiology |
| PMN             | Class II       | 21 CFR 866.3920 – Assayed quality control material for clinical microbiology assays                        | MI - Microbiology |

### II Submission/Device Overview:

#### A Purpose for Submission:

To obtain a substantial equivalence determination for the BioFire Global Fever Special Pathogens Panel and the BioFire SHIELD Control Kit for the BioFire Global Fever Special Pathogens Panel.

## **B Measurand:**

DNA and RNA sequences from the following organisms: Chikungunya virus, Dengue virus serotypes 1-4, *Leishmania* spp. (*Leishmania donovani* and *Leishmania infantum*), *Leptospira* spp., *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi*, West Nile virus, *Bacillus anthracis*, Crimean-Congo hemorrhagic fever virus, *Ebolavirus* spp., *Francisella tularensis*, Lassa virus, *Marburgvirus*, Yellow fever virus, and *Yersinia pestis*.

## **C Type of Test:**

Multiplex Nucleic Acid Amplification Test

## **III Intended Use/Indications for Use:**

### **A Intended Use(s):**

See Indications for Use below.

### **B Indication(s) for Use:**

The BioFire Global Fever Special Pathogens Panel is a qualitative, multiplexed, nucleic acid-based test intended for use with BioFire FilmArray 2.0 and BioFire FilmArray Torch Systems. The BioFire Global Fever Special Pathogens Panel is for the simultaneous qualitative detection and identification of multiple bacterial, viral, and protozoan nucleic acids directly from EDTA whole blood collected from individuals with signs and/or symptoms of acute febrile illness or recent acute febrile illness and known or suspected exposure to the target pathogens described below.

Pathogens identified:

Chikungunya virus

Dengue virus (serotypes 1, 2, 3 and 4)

*Leishmania* spp. that cause visceral leishmaniasis (e.g., *L. donovani* and *L. infantum*)

*Leptospira* spp.

*Plasmodium* spp. (including species differentiation of *Plasmodium falciparum* and *Plasmodium vivax/ovale*)

West Nile virus

Pathogens presumptively identified:

*Bacillus anthracis*

Crimean-Congo hemorrhagic Fever virus

*Ebolavirus* spp.

*Francisella tularensis*

Lassa virus

*Marburgvirus*

Yellow fever virus

*Yersinia pestis*

Pathogens for which the panel provides presumptive identification results require additional testing and confirmation procedures in consultation with the appropriate public health authorities for whom reports may be necessary.

Positive results do not rule out co-infections with pathogens not included on the BioFire Global Fever Special Pathogens Panel. Not all pathogens that cause acute febrile illness are detected by this test, and negative results do not preclude infection with the pathogens targeted by the device and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

Evaluation for more common causes of acute febrile illness (e.g., infections of the upper and lower respiratory tract or gastroenteritis, as well as non-infectious causes) should be considered prior to evaluation with this panel. In the United States, patient travel history, exposure risk, and consultation of the CDC Yellow Book should be considered prior to use of the BioFire Global Fever Special Pathogens Panel as some pathogens are more common in certain geographical locations. Results are meant to be used in conjunction with other clinical, epidemiologic, and laboratory data, in accordance with the guidelines provided by the relevant public health authorities.

The BioFire Global Fever Special Pathogens Panel is indicated for use in laboratories having appropriate biosafety equipment, personal protective equipment (PPE), containment facilities and personnel trained in the safe handling of diagnostic clinical specimens potentially containing any of the pathogens detected by this panel.

The BioFire Global Fever Special Pathogens Panel is indicated for use in laboratories that follow public health guidelines that address appropriate biosafety conditions, interpretation of test results, and coordination of findings with public health authorities.

#### For In Vitro Diagnostic Use

The BIOFIRE SHIELD Control Kit for the BioFire Global Fever Special Pathogens Panel contains Positive and Negative External Controls intended for use as assayed quality controls to monitor the performance of in vitro diagnostic laboratory nucleic acid testing procedures for the qualitative detection of *Bacillus anthracis*, chikungunya virus, Crimean-Congo hemorrhagic fever virus, dengue virus (serotypes 1, 2, 3, and 4), *Ebolavirus* spp., *Francisella tularensis*, Lassa virus, *Leishmania* spp., *Leptospira* spp., *Marburgvirus*, *Plasmodium* spp. (including species differentiation of *Plasmodium falciparum* and *Plasmodium vivax/ovale*), West Nile virus, Yellow fever virus, and *Yersinia pestis* when using the BioFire Global Fever Special Pathogens Panel on BioFire FilmArray 2.0 and BioFire FilmArray Torch Systems. The BIOFIRE SHIELD Control Kit for the BioFire Global Fever Special Pathogens Panel is designed for and intended to be used solely with the BioFire Global Fever Special Pathogens Panel. This product does not replace manufacturer internal controls provided as part of the BioFire Global Fever Special Pathogens Panel.

Both the Positive and Negative External Controls are provided in a FilmArray Control Injection Vial format. The Positive Control Injection Vial contains dried synthetic DNA segments in buffer and stabilizer to assess the presence of each individual assay on the BioFire Global Fever

Special Pathogens Panel. The Negative Control Injection Vial contains no DNA and is non-reactive with the BioFire Global Fever Special Pathogens Panel assays.

**C Special Conditions for Use Statement(s):**

Rx - For Prescription Use Only

**D Special Instrument Requirements:**

The FilmArray Global Fever Special Pathogens Panel is performed on the FilmArray 2.0 and FilmArray Torch systems.

**IV Device/System Characteristics:**

**A Device Description:**

The BioFire Global Fever Special Pathogens Panel is a multiplex nucleic acid-based test designed to be used with the FilmArray 2.0 or the FilmArray Torch instruments. The BioFire Global Fever Special Pathogens Panel includes a pouch which contains freeze-dried reagents to perform nucleic acid purification and nested, multiplex polymerase chain reaction (PCR) with DNA melt analysis. The BioFire Global Fever Special Pathogens Panel simultaneously conducts sixteen tests for the identification of bacterial, viral, and protozoan organisms from whole blood specimens collected in EDTA tubes. Results from the BioFire Global Fever Special Pathogens Panel are available within about one hour.

A test is initiated by loading Hydration Solution into one port of the pouch and a whole blood specimen mixed with the provided Sample Buffer into the other port of the pouch and placing it in the FilmArray Instrument. The pouch contains all of the reagents required for specimen testing and analysis in a freeze-dried format; the addition of Hydration Solution and the Sample Buffer rehydrates the reagents. After the pouch is prepared, the FilmArray Software on the FilmArray system guides the user through the steps of placing the pouch into the instrument, scanning the pouch barcode, entering the sample identification, selecting the appropriate protocol, and initiating the run on the FilmArray system.

The FilmArray instruments contain a coordinated system of inflatable bladders and seal points, which act on the pouch to control the movement of liquid between the pouch blisters. When a bladder is inflated over a reagent blister, it forces liquid from the blister into connecting channels. Alternatively, when a seal is placed over a connecting channel it acts as a valve to open or close a channel. In addition, electronically controlled pneumatic pistons are positioned over multiple plungers in order to deliver the rehydrated reagents into the blisters at the appropriate times. Two Peltier devices control heating and cooling of the pouch to drive the PCR reactions and the melt curve analysis.

Nucleic acid extraction occurs within the BioFire pouch using mechanical and chemical lysis followed by purification using standard magnetic bead technology. After extracting and purifying nucleic acids from the unprocessed sample, a nested multiplex PCR is executed in two stages. During the first stage, a single, large volume, highly multiplexed reverse transcription PCR (RT-PCR) reaction is performed. The products from first stage PCR are then diluted and combined with a fresh, primer-free master mix and a fluorescent double stranded DNA binding dye (LC Green Plus, BioFire Defense, LLC). The solution is then

distributed to each well of the array. Array wells contain sets of primers designed specifically to amplify sequences internal to the PCR products generated during the first stage PCR reaction. The 2<sup>nd</sup> stage PCR, or nested PCR, is performed in each well of the array. At the conclusion of the 2<sup>nd</sup> stage PCR, the array is interrogated by melt curve analysis for the detection of signature amplicons denoting the presence of specific targets. A digital camera placed in front of the array captures fluorescent images of the PCR2 reactions and software interprets the data.

The FilmArray instrument software automatically interprets the results of each DNA melt curve analysis and combines the data with the results of the internal pouch controls to provide a test result for each organism on the panel. A description of the individual assays and their result interpretation is included below:

- Chikungunya virus: The Global Fever Special Pathogens Panel contains two assays for species-level detection of all chikungunya virus strains (CHIKV1 and CHIKV2). The FilmArray instrument software will interpret any single positive chikungunya assay as a Chikungunya Virus Detected result.
- Dengue virus: The Global Fever Special Pathogens Panel contains five individual assays for the detection of Dengue virus serotypes 1, 2, 3, and 4 with two assays specifically dedicated to detecting Dengue virus serotype 2 (DENV1, DENV2\_1, DENV2\_2, DENV3, and DENV4). The FilmArray instrument software will interpret any single positive Dengue virus assay as a Dengue Virus Detected result.
- Leptospira: The Global Fever Special Pathogens Panel contains a single pan assay for the genus-level detection of all *Leptospira* Group 1 species (LEPTO1). A positive pan-*Leptospira* assay will result in a *Leptospira* spp. Detected call.
- Leishmania spp.: The Global Fever Special Pathogens panel contains a single assay for the genus-level detection of *Leishmania*. The assay detects species that cause visceral leishmaniasis (*L. infantum* and *L. donovani*). Other species of *Leishmania* are expected to be generate a detected result. A positive *Leishmania* assay will result in a Detected *Leishmania* spp. test result.
- Plasmodium: The Global Fever Special Pathogens Panel contains three *Plasmodium* assays, one genus-level assay and two species-level assays. The genus-level assay (*Plasmodium* spp.) detects all five *Plasmodium* species known to infect humans (*P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*). One species-level assay detects *Plasmodium falciparum* and a combined species-level assay detects both *Plasmodium vivax* and *Plasmodium ovale*. Each individual assay is reported as a Detected or Not Detected result separately based on the results of the specific Global Fever Special Pathogens Panel assay.
- Bacillus anthracis: The Global Fever Special Pathogens Panel contains one assay for the detection of *Bacillus anthracis*. a positive *Bacillus anthracis* assay will result in a *Bacillus anthracis* Detected result.

- *Francisella tularensis*: The Global Fever Special Pathogens Panel contains two assays for the detection of *Francisella tularensis*. One or more positive *Francisella tularensis* assays will result in a Detected call for *Francisella tularensis*.
- *Yersinia pestis*: The Global Fever Special Pathogens Panel contains two assays for the detection of *Yersinia pestis*. One or more positive *Yersinia pestis* assays will result in a Detected call for *Yersinia pestis*.
- Crimean-Congo hemorrhagic fever virus: The Global Fever Special Pathogens Panel contains two assays for the detection of Crimean-Congo hemorrhagic fever virus. One or more positive Crimean-Congo hemorrhagic fever virus assays will result in a Detected call for Crimean-Congo hemorrhagic fever virus.
- *Ebolavirus* spp.: The Global Fever Special Pathogens Panel contains three assays for the detection of *Ebolavirus* spp. Any positive assay call will result in a Detected call for *Ebolavirus*.
- Lassa virus: The Global Fever Special Pathogens Panel contains two assays for the detection of Lassa virus. Any positive assay call will result in a Detected call for Lassa virus.
- *Marburgvirus*: The Global Fever Special Pathogens Panel contains one assay for the detection of *Marburgvirus*. A positive assay call will result in a *Marburgvirus* Detected result.
- Yellow fever virus: The Global Fever Special Pathogens Panel contains one assay for the detection of Yellow Fever virus. A positive assay call will result in a Yellow Fever virus Detected result.

Materials provided in each BioFire Global Fever Special Pathogens Panel kit:

- Individually packaged BioFire Global Fever Panel pouches
- Individually-packaged Transfer Pipettes
- Single-use (1.0 mL) BioFire FilmArray Sample Buffer Tubes
- Single-use pre-filled (1.5 mL) BioFire FilmArray Hydration Injection Vials (Blue)
- Single-use BioFire FilmArray Sample Injection Vials (Red)
- Instructions and Documents
  - BioFire Global Fever Special Pathogens Panel Instructions for Use
  - BioFire Global Fever Special Pathogens Panel Quick Guide

Materials required but not provided:

- 10% bleach solution

BioFire FilmArray system including:

- BioFire FilmArray 2.0 or FilmArray Torch instrument, computer, and software
- FilmArray Pouch Loading Station

## B Principle of Operation:

The BioFire Global Fever Special Pathogens Panel pouch is a closed system disposable that houses all the chemistry required to isolate, amplify, and detect nucleic acid from multiple biothreat pathogens within whole blood samples. The rigid plastic component (fitment) of the pouch contains reagents in freeze-dried form. The flexible plastic portion of the pouch is divided into discrete segments (blisters) where the required chemical processes are carried out. The user of the BioFire Global Fever Special Pathogens Panel loads the sample into the pouch, places the pouch into the FilmArray instrument, and starts the run. All other operations are automated. Operations and processes that occur during a FilmArray run include the following:

- **Nucleic Acid Purification-** Nucleic acid purification occurs in the first three blisters of the pouch. The sample is lysed by agitation (bead beating) and the liberated nucleic acid is captured, washed, and eluted using magnetic bead technology. These steps require about ten minutes and the bead-beater apparatus can be heard as a high-pitched whine during the first minute of operation.
- **Reverse Transcription and 1<sup>st</sup> Stage Multiplex PCR** - Some pathogens identified by the FilmArray Global Fever pouch are RNA viruses, and a reverse transcription (RT) step is performed to convert the viral RNA into cDNA prior to amplification. The purified nucleic acid solution is combined with a preheated master mix to initiate the RT step and subsequent thermocycling for multiplex PCR. The effect of 1<sup>st</sup> stage PCR is to enrich for the target nucleic acids present in the sample.
- **2<sup>nd</sup> Stage PCR** - The products of 1<sup>st</sup> stage PCR are diluted and mixed with fresh PCR reagents containing an intercalating fluorescent DNA dye (LCGreen Plus, BioFire Defense, LLC). This solution is distributed over the 2<sup>nd</sup> stage PCR array. The individual wells of the array contain primers for different assays (each present in triplicate) that target specific nucleic acid sequences from each of the pathogens detected, as well as control template material. These primers are “nested” or internal to the specific products of the 1<sup>st</sup> stage multiplex reaction, which enhances both the sensitivity and specificity of the reactions.
- **DNA Melting Analysis** – After 2<sup>nd</sup> stage PCR, the temperature is slowly increased and fluorescence in each well of the array is monitored and analyzed to generate a melt curve. The temperature at which a specific PCR product melts (melting temperature or T<sub>m</sub>) is consistent and predictable and the FilmArray software automatically evaluates the data from replicate wells for each assay to report results.

The FilmArray software controls the operation of the instrument, collects and analyzes data and automatically generates a test report at the end of the run.

## V Substantial Equivalence Information:

### A Predicate Device Name(s):

FilmArray NGDS Warrior Panel, FilmArray Global Fever Panel, BIOFIRE SHIELD Control Kit for the Global Fever Panel

### B Predicate 510(k) Number(s):

DEN160048, DEN200043, K202382

### C Comparison with Predicate(s):

| Device & Predicate Device(s):              | <u>K213362</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | <u>DEN160048</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | <u>DEN200043</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|--------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Device Trade Name                          | BioFire Global Fever Special Pathogens Panel                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | FilmArray NGDS Warrior Panel                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | FilmArray Global Fever Panel                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| General Device Characteristic Similarities |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Intended Use/Indications For Use           | <p>The BioFire Global Fever Special Pathogens Panel is a qualitative, multiplexed, nucleic acid-based test intended for use with BioFire FilmArray 2.0 and BioFire FilmArray Torch Systems. The BioFire Global Fever Special Pathogens Panel is for the simultaneous qualitative detection and identification of multiple bacterial, viral, and protozoan nucleic acids directly from EDTA whole blood collected from individuals with signs and/or symptoms of acute febrile illness or recent acute febrile illness and known or suspected exposure to the target pathogens described below.</p> <p>Pathogens identified:</p> | <p>The FilmArray NGDS Warrior Panel is a qualitative, multiplexed, nucleic acid-based in vitro diagnostic test intended for use with the FilmArray 2.0 system. The FilmArray NGDS Warrior Panel detects and identifies <i>Bacillus anthracis</i>, <i>Yersinia pestis</i>, <i>Francisella tularensis</i>, <i>Coxiella burnetii</i>, Ebola virus, and Marburg virus nucleic acids directly from human whole blood (EDTA). The FilmArray NGDS Warrior Panel is also intended to be used to test for <i>Bacillus anthracis</i> or <i>Yersinia pestis</i> nucleic acids in blood cultures that are determined to be positive either by an automated system, by turbidity, or by daily Gram stain even</p> | <p>The FilmArray Global Fever Panel is a qualitative, multiplexed, nucleic acid-based in vitro diagnostic test intended for use with the FilmArray 2.0 system. The FilmArray Global Fever Panel detects and identifies selected bacterial, viral, and protozoan nucleic acids directly from EDTA whole blood collected from individuals with signs and/or symptoms of acute febrile illness or recent acute febrile illness and known or suspected exposure to the following target pathogens: <i>Leptospira</i> spp., chikungunya virus, dengue virus (serotypes 1, 2, 3 and 4), and <i>Plasmodium</i> spp. (including species differentiation of <i>Plasmodium falciparum</i></p> |

|  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|--|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  | <p>Chikungunya virus<br/>Dengue virus (serotypes 1, 2, 3 and 4)<br/><i>Leishmania</i> spp. that cause visceral leishmaniasis (e.g., <i>L. donovani</i> and <i>L. infantum</i>)<br/><i>Leptospira</i> spp.<br/><i>Plasmodium</i> spp. (including species differentiation of <i>Plasmodium falciparum</i> and <i>Plasmodium vivax/ovale</i>)<br/>West Nile virus</p> <p>Pathogens presumptively identified:</p> <p><i>Bacillus anthracis</i><br/>Crimean-Congo Hemorrhagic Fever virus<br/><i>Ebolavirus</i> spp.<br/><i>Francisella tularensis</i><br/>Lassa virus<br/>Marburgvirus<br/>Yellow Fever virus<br/><i>Yersinia pestis</i></p> <p>Pathogens for which the panel provides presumptive identification results require additional testing and confirmation procedures in consultation with the appropriate public health authorities for whom reports may be necessary.</p> <p>Positive results do not rule out co-infections with pathogens not</p> | <p>without turbidity, and is indicated to be performed with concomitant Gram stain performed on positive blood culture specimens as per normal laboratory procedure.</p> <p>The FilmArray NGDS Warrior Panel is intended to test individuals with signs and symptoms of infection from biothreat agents and/or individuals who are at risk for exposure or may have been exposed to these agents.</p> <p>The FilmArray NGDS Warrior Panel is indicated as an aid in the diagnosis of anthrax, plague, tularemia, Q fever, and the hemorrhagic fevers caused by Ebola and Marburg viruses, in response to a suspected or confirmed bioterrorism event or outbreaks. It is for diagnostic use in conjunction with other clinical, epidemiologic, and laboratory data, in accordance with the guidelines provided by the appropriate Department of Defense and public health authorities.</p> <p>Results are for the presumptive</p> | <p>and <i>Plasmodium vivax/ovale</i>).<br/>Evaluation for more common causes of acute febrile illness (e.g., infections of the upper and lower respiratory tract or gastroenteritis, as well as non-infectious causes) should be considered prior to evaluation with this panel. Results are meant to be used in conjunction with other clinical, epidemiologic, and laboratory data, in accordance with the guidelines provided by the relevant public health authorities.</p> <p>Positive results do not rule out co-infections with pathogens not included on the FilmArray Global Fever Panel. Not all pathogens that cause acute febrile illness are detected by this test, and negative results do not rule out the presence of other infections. Patient travel history and consultation of the CDC Yellow Book should be considered prior to use of the FilmArray Global Fever Panel as some pathogens are more common in certain geographical locations.</p> |
|--|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

|  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |  |
|--|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|
|  | <p>included on the BioFire Global Fever Special Pathogens Panel. Not all pathogens that cause acute febrile illness are detected by this test, and negative results do not preclude infection with the pathogens targeted by the device and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.</p> <p>Evaluation for more common causes of acute febrile illness (e.g., infections of the upper and lower respiratory tract or gastroenteritis, as well as non-infectious causes) should be considered prior to evaluation with this panel. In the United States, patient travel history, exposure risk, and consultation of the CDC Yellow Book should be considered prior to use of the BioFire Global Fever Special Pathogens Panel as some pathogens are more common in certain geographical locations. Results are meant to be used in conjunction with other clinical, epidemiologic, and laboratory data, in accordance with the guidelines provided by the relevant public health authorities.</p> | <p>identification of <i>Bacillus anthracis</i>, <i>Yersinia pestis</i>, <i>Francisella tularensis</i>, <i>Coxiella burnetii</i>, Ebola virus, and Marburg virus. The definitive identification of <i>Bacillus anthracis</i>, <i>Yersinia pestis</i>, <i>Francisella tularensis</i>, <i>Coxiella burnetii</i>, Ebola virus, and Marburg virus requires additional testing and confirmation procedures in consultation with the appropriate Department of Defense and public health authorities for whom reports may be necessary. Negative results do not preclude infection with these biothreat agents and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.</p> <p>The FilmArray NGDS Warrior Panel is solely for use by United States Department of Defense laboratories, and laboratories designated by the Department of Defense.</p> |  |
|--|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|

|                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |      |                                                                         |
|--------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|-------------------------------------------------------------------------|
|                          | <p>The BioFire Global Fever Special Pathogens Panel is indicated for use in laboratories having appropriate biosafety equipment, personal protective equipment (PPE), containment facilities and personnel trained in the safe handling of diagnostic clinical specimens potentially containing any of the pathogens detected by this panel.</p> <p>The BioFire Global Fever Special Pathogens Panel is indicated for use in laboratories that follow public health guidelines that address appropriate biosafety conditions, interpretation of test results, and coordination of findings with public health authorities.</p> <p>For In Vitro Diagnostic Use.</p> |      |                                                                         |
| Specimen Type            | Whole blood (collected in EDTA tube)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Same | Whole Blood (collected in EDTA tube), positive blood culture, or sputum |
| Analyte                  | RNA/DNA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Same | Same                                                                    |
| Technological Principles | Multiplexed nested nucleic acid amplification test with melt analysis                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Same | Same                                                                    |
| Time to result           | About 1 hour                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Same | Same                                                                    |
| Reagent storage          | Room temperature                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Same | Same                                                                    |
| Test interpretation      | Automated test interpretation and report                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Same | Same                                                                    |

|                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                |                                                                                                                                                                                                                      |
|--------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                  | generation. User cannot access raw data.                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                                                |                                                                                                                                                                                                                      |
| Controls                                         | Two controls included in each reagent pouch to control for sample processing and both stages of PCR and melt analysis                                                                                                                                                                                                                                                                                                                                                                                  | Same                                                                                                                                           | Same                                                                                                                                                                                                                 |
| <b>General Device Characteristic Differences</b> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                |                                                                                                                                                                                                                      |
| Pathogens detected                               | <i>Bacillus anthracis</i> , Crimean-Congo hemorrhagic fever virus, Ebolavirus spp., <i>Francisella tularensis</i> , Lassa virus, <i>Leishmania</i> spp. ( <i>L. donovani</i> and <i>L. infantum</i> ), <i>Leptospira</i> spp., Marburgvirus, chikungunya virus, dengue virus (serotypes 1, 2, 3 and 4), and <i>Plasmodium</i> spp. (including species differentiation of <i>Plasmodium falciparum</i> and <i>Plasmodium vivax/ovale</i> ), West Nile virus, Yellow fever virus, <i>Yersinia pestis</i> | <i>Bacillus anthracis</i> , <i>Yersinia pestis</i> , <i>Francisella tularensis</i> , <i>Coxiella burnetii</i> , Ebola virus, and Marburg virus | <i>Leptospira</i> spp., chikungunya virus, dengue virus (serotypes 1, 2, 3 and 4), and <i>Plasmodium</i> spp. (including species differentiation of <i>Plasmodium falciparum</i> and <i>Plasmodium vivax/ovale</i> ) |
| User Complexity                                  | High                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Moderate                                                                                                                                       | Moderate                                                                                                                                                                                                             |

### Comparison with BIOFIRE SHIELD Control Kit for the Global Fever Panel

| Device & Predicate Device(s):                     | <u>K213362</u>                                                                  | <u>K202382</u>                                                             |
|---------------------------------------------------|---------------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Device Trade Name                                 | BIOFIRE SHIELD Control Kit for the BioFire Global Fever Special Pathogens Panel | BIOFIRE SHIELD Control Kit for the Global Fever Panel                      |
| <b>General Device Characteristic Similarities</b> |                                                                                 |                                                                            |
| Intended Use/Indications For Use                  | The BIOFIRE SHIELD Control Kit for the BioFire Global Fever Special             | The BIOFIRE SHIELD Control Kit for the BioFire Global Fever Panel contains |

|  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|--|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  | <p>Pathogens Panel contains Positive and Negative External Controls intended for use as assayed quality controls to monitor the performance of <i>in vitro</i> diagnostic laboratory nucleic acid testing procedures for the qualitative detection of <i>Bacillus anthracis</i>, chikungunya virus, Crimean-Congo hemorrhagic fever virus, dengue virus (serotypes 1, 2, 3, and 4), <i>Ebolavirus</i> spp., <i>Francisella tularensis</i>, Lassa virus, <i>Leishmania</i> spp., <i>Leptospira</i> spp., <i>Marburgvirus</i>, <i>Plasmodium</i> spp. (including species differentiation of <i>Plasmodium falciparum</i> and <i>Plasmodium vivax/ovale</i>), West Nile virus, yellow fever virus, and <i>Yersinia pestis</i> when using the BioFire® Global Fever Special Pathogens Panel on BioFire FilmArray 2.0 and BioFire FilmArray Torch Systems. The BIOFIRE SHIELD Control Kit for the BioFire Global Fever Special Pathogens Panel is designed for and intended to be used solely with the BioFire Global Fever Special Pathogens Panel. This product does not replace manufacturer internal controls provided as part of the BioFire Global Fever Special Pathogens Panel.</p> <p>Both the Positive and Negative External Controls are provided in a FilmArray Control Injection Vial format. The Positive Control Injection Vial contains dried synthetic DNA segments in buffer and stabilizer to assess the</p> | <p>Positive and Negative External Controls intended for use as assayed quality controls to monitor the performance of <i>in vitro</i> diagnostic laboratory nucleic acid testing procedures for the qualitative detection of chikungunya virus, dengue virus (serotypes 1, 2, 3, and 4), <i>Leptospira</i> spp., and <i>Plasmodium</i> spp. (including species differentiation of <i>Plasmodium falciparum</i> and <i>Plasmodium vivax/ovale</i>) when using the BioFire Global Fever Panel on BioFire FilmArray 2.0 and BioFire FilmArray Torch Systems. The BIOFIRE SHIELD Control Kit for the BioFire Global Fever Panel is designed for and intended to be used solely with the BioFire Global Fever Panel. This product does not replace manufacturer internal controls provided as part of the BioFire Global Fever Panel device.</p> <p>Both the Positive and Negative External Controls are provided in a FilmArray Control Injection Vial format. The Positive Control Injection Vial contains dried synthetic DNA segments in buffer and stabilizer to assess the presence of each individual assay on the BioFire Global Fever Panel. The Negative Control Injection Vial contains no DNA and is non-reactive with the BioFire Global Fever Panel assays.</p> |
|--|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

|                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                         |
|--------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                  | <p>presence of each individual assay on the BioFire Global Fever Special Pathogens Panel. The Negative Control Injection Vial contains no DNA and is non-reactive with the BioFire Global Fever Special Pathogens Panel assays.</p> <p>For In Vitro Diagnostic Use.</p>                                                                                                                                                                                                                                  |                                                                                                                                                                                                         |
| Device Description                               | Positive and negative external assayed quality controls to monitor assay performance                                                                                                                                                                                                                                                                                                                                                                                                                     | Same                                                                                                                                                                                                    |
| Physical Format                                  | External control material dried on Control Injection Filter                                                                                                                                                                                                                                                                                                                                                                                                                                              | Same                                                                                                                                                                                                    |
| Composititon                                     | Tm-shifted synthetic DNA (positive control only)                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Same                                                                                                                                                                                                    |
| Time to result                                   | About 1 hour                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Same                                                                                                                                                                                                    |
| Reagent storage                                  | Room temperature                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Same                                                                                                                                                                                                    |
| Test interpretation                              | Automated test interpretation and report generation. User cannot access raw data.                                                                                                                                                                                                                                                                                                                                                                                                                        | Same                                                                                                                                                                                                    |
| Instrumentation                                  | BioFire FilmArray 2.0 or BioFire FilmArray Torch systems                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Same                                                                                                                                                                                                    |
| <b>General Device Characteristic Differences</b> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                         |
| Targets Monitored                                | <p><i>Bacillus anthracis</i>, Crimean-Congo hemorrhagic fever virus, Ebolavirus spp., <i>Francisella tularensis</i>, Lassa virus, <i>Leishmania</i> spp. (<i>L. donovani</i> and <i>L. infantum</i>), <i>Leptospira</i> spp., Marburgvirus, chikungunya virus, dengue virus (serotypes 1, 2, 3 and 4), and <i>Plasmodium</i> spp. (including species differentiation of <i>Plasmodium falciparum</i> and <i>Plasmodium vivax/ovale</i>), West Nile virus, Yellow fever virus, <i>Yersinia pestis</i></p> | <p>Chikungunya virus, dengue virus (serotypes 1, 2, 3, and 4), <i>Leptospira</i> spp., <i>Plasmodium</i> spp. (including species differentiation of <i>P. falciparum</i> and <i>P. vivax/ovale</i>)</p> |

## **VI Standards/Guidance Documents Referenced:**

14971:2007/(R)2010 (Corrected 4 October 2007), ‘Medical Devices – Applications of Risk Management to Medical Devices’

Guidance for Industry and Food and Drug Administration Staff – De Novo Classification Process (Evaluation of Automatic Class III Designation) (October 30, 2017)

Guidance for Industry and Food and Drug Administration Staff – Highly Multiplexed Microbiological/Medical Countermeasure In Vitro Nucleic Acid Based Diagnostic Devices (August 27, 2014)

Guideline for Industry and Food and Drug Administration Staff – Class II Special Controls Guideline: Dengue Virus Nucleic Acid Amplification Test Reagents

Guideline for Industry and Food and Drug Administration Staff – Class II Special Controls Guidance Document: Plasmodium Species Antigen Detection Assays

Guidance for Industry and FDA Staff – Assayed and Unassayed Quality Control Material

Guidance for Industry and FDA Staff – Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests (March 13, 2007)

MM03-Ed3, Molecular Diagnostic Methods for Infectious Diseases, CLSI Approved Guideline—Third Edition

EP07-Ed3, Interference Testing in Clinical Chemistry; Approved Guideline—Third Edition

Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices, FDA Guidance Document

Guidance for Industry, FDA Reviewers and Compliance on Off-The-Shelf Software Use in Medical Devices

Guidance for Industry, FDA Reviewers and Compliance on Off-The-Shelf Software Use in Medical Devices

ISO 62304:2006, ‘Medical Device Software – Software Life Cycle Processes’ – IEC 62304:2006, November 27, 2008

ISO 62304:2006, ‘Medical Device Software – Software Life Cycle Processes’ – IEC 62304:2006, November 27, 2008

ISO 15223-1:2012, ‘Medical Devices – Symbols to be used with medical device labels, labeling and information to be supplied – Part 1: General requirements’

## VII Performance Characteristics (if/when applicable):

### A Analytical Performance:

#### 1. Precision/Reproducibility:

To evaluate the reproducibility of the BioFire Global Fever Special Pathogens Panel, three contrived whole blood samples were prepared with different mixtures of representative panel analytes. For each analyte, one sample was spiked at a Moderate Positive concentration (3 x LoD), another sample at a Low Positive (1 x LoD) and a third sample that was negative (unspiked) for the given analyte. For *P. falciparum*, a dilution error occurred that resulted in lower than expected analyte concentration being evaluated for all replicates. Six replicates of each sample were tested across 3 different sites on five different days, providing a total of 90 replicate test results per sample. On each test day at each site, two different operators used three FilmArray 2.0 instruments; GF Panel pouch lot was rotated daily. In total, 270 valid test results were obtained for the reproducibility evaluation of the BioFire Global Fever Special Pathogens Panel.

**Table 1. Contrived Whole Blood Samples for Reproducibility Testing**

| Organism (Isolate)                                              | Sample 1                                            | Sample 2                                          | Sample 3                                            |
|-----------------------------------------------------------------|-----------------------------------------------------|---------------------------------------------------|-----------------------------------------------------|
| <i>Leptospira interrogans</i><br>serovar<br>icterohaemorrhagiae | Low Positive<br>3.3E+02 copies/mL<br>1 x LoD        | Moderate Positive<br>1.0E+03 copies/mL<br>3 x LoD | Negative                                            |
| <b>Dengue Virus (DENV-2)</b><br>New Guinea C                    | Low Positive<br>3.3E+02 copies/mL<br>1 x LoD        | Moderate Positive<br>1.0E+03 copies/mL<br>3 x LoD | Negative                                            |
| <i>Plasmodium falciparum</i><br>IPC 4884                        | Moderate Positive<br>5.4E+02 copies/mL<br>1.5 x LoD | Negative                                          | Low Positive<br>9.0E+01 copies/mL<br>0.5 x LoD      |
| <i>Leishmania donovani</i>                                      | None                                                | Low Positive<br>1.1E+01 copies/mL<br>1.1 x LoD    | Moderate Positive<br>3.4E+01 copies/mL<br>3.4 x LoD |

Combined reproducibility results are shown below in **Table 2**.

**Table 2: Reproducibility of the FilmArray Global Fever Special Pathogens Panel**  
**Qualitative Results**

|                                                                                                               | Test Analyte Isolate                                                   |                                                   | Concentration                                                     | Expected Test Result | % Agreement with Expected Results |                |                |                |                         |
|---------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------|---------------------------------------------------|-------------------------------------------------------------------|----------------------|-----------------------------------|----------------|----------------|----------------|-------------------------|
|                                                                                                               |                                                                        |                                                   |                                                                   |                      | Site 1                            | Site 2         | Site 3         | All Sites      | 95% Confidence Interval |
| BACTERIA                                                                                                      | <i>Leptospira interrogans</i><br>serovar<br><i>icterohaemorrhagiae</i> |                                                   | Moderate Positive<br>3× LoD<br>(1.2E+03 copies/mL)                | Detected             | 30/30<br>100%                     | 30/30<br>100%  | 30/30<br>100%  | 90/90<br>100%  | 96.0-100%               |
|                                                                                                               |                                                                        |                                                   | Low Positive<br>1× LoD<br>(3.9E+02 copies/mL)                     | Detected             | 27/30<br>90.0%                    | 28/30<br>93.4% | 26/30<br>86.7% | 81/90<br>90.0% | 81.9-95.3%              |
|                                                                                                               |                                                                        |                                                   | Negative<br>(No Analyte)                                          | Not Detected         | 30/30<br>100%                     | 30/30<br>100%  | 30/30<br>100%  | 90/90<br>100%  | 96.0-100%               |
| VIRUSES                                                                                                       | Dengue virus<br><br>DENV-2<br><br>New Guinea C                         |                                                   | Moderate Positive<br>3× LoD<br>(1.1E+03 copies/mL)                | Detected             | 29/30<br>96.7%                    | 30/30<br>100%  | 30/30<br>100%  | 89/90<br>98.9% | 94.0-100%               |
|                                                                                                               |                                                                        |                                                   | Low Positive<br>1× LoD<br>(3.6E+02 copies/mL)                     | Detected             | 30/30<br>100%                     | 30/30<br>100%  | 30/30<br>100%  | 90/90<br>100%  | 96.0-100%               |
|                                                                                                               |                                                                        |                                                   | Negative<br>(No Analyte)                                          | Not Detected         | 30/30<br>100%                     | 30/30<br>100%  | 30/30<br>100%  | 90/90<br>100%  | 96.0-100%               |
| PROTOZOA                                                                                                      | <i>Plasmodium falciparum</i><br><br>IPC 4884                           | <i>Plasmodium</i> spp.<br>Detection Results       | Moderate Positive<br>1.5× LoD <sup>1</sup><br>(2.7E+02 copies/mL) | Detected             | 30/30<br>100%                     | 30/30<br>100%  | 30/30<br>100%  | 90/90<br>100%  | 96.0-100%               |
|                                                                                                               |                                                                        |                                                   | Low Positive<br>0.5× LoD <sup>1</sup><br>(9.0E+01 copies/mL)      | Detected             | 28/30<br>93.3%                    | 30/30<br>100%  | 29/30<br>96.7% | 87/90<br>96.7% | 90.7-98.9%              |
|                                                                                                               |                                                                        |                                                   | Negative<br>(No Analyte)                                          | Not Detected         | 30/30<br>100%                     | 30/30<br>100%  | 30/30<br>100%  | 90/90<br>100%  | 96.0-100%               |
|                                                                                                               |                                                                        | <i>Plasmodium falciparum</i><br>Detection Results | Moderate Positive<br>1.5× LoD <sup>1</sup><br>(2.7E+02 copies/mL) | Detected             | 29/30<br>96.7%                    | 30/30<br>100%  | 28/30<br>93.4% | 87/90<br>96.7% | 90.6-99.3%              |
|                                                                                                               |                                                                        |                                                   | Low Positive<br>0.5× LoD <sup>1</sup><br>(9.0E+01 copies/mL)      | Detected             | 18/30<br>60%                      | 24/30<br>80%   | 21/30<br>70%   | 63/90<br>70.0% | 59.4-79.2%              |
|                                                                                                               |                                                                        |                                                   | Negative<br>(No Analyte)                                          | Not Detected         | 30/30<br>100%                     | 30/30<br>100%  | 30/30<br>100%  | 90/90<br>100%  | 96.0-100%               |
|                                                                                                               | <i>Leishmania donovani</i><br><br>1S (MHOM/SD/62/1S)                   |                                                   | Moderate Positive<br>3.4 × LoD<br>(3.4E+01 copies/mL)             | Detected             | 30/30<br>100%                     | 30/30<br>100%  | 30/30<br>100%  | 90/90<br>100%  | 96.0-100%               |
|                                                                                                               |                                                                        |                                                   | Low Positive<br>1.1 × LoD <sup>1</sup><br>(1.1E+01 copies/mL)     | Detected             | 30/30<br>100%                     | 30/30<br>100%  | 30/30<br>100%  | 90/90<br>100%  | 96.0-100%               |
|                                                                                                               |                                                                        |                                                   | Negative<br>(No Analyte)                                          | Not Detected         | 30/30<br>100%                     | 30/30<br>100%  | 30/30<br>100%  | 90/90<br>100%  | 96.0-100%               |
| Overall Agreement with the Expected Test Result<br>All Analytes and All Test Levels (95% Confidence Interval) |                                                                        |                                                   |                                                                   |                      | 1,307/1,350<br>96.8% (95.7-97.6%) |                |                |                |                         |

<sup>1</sup>Due to a correction in the stock concentration, *P. falciparum* was evaluated at 1.5x LoD and 0.5x LoD.

Detected results were as expected for all analytes except for the Low Positive *Leptospira interrogans* sample which exhibited 90% agreement. The observed negative specimens were distributed across all runs, sites, testing days, and reagent lots and likely reflect underspiking with *Leptospira interrogans*.

A secondary assessment of reproducibility is based on variability in the melt temperature (T<sub>m</sub>) of the amplification products (measured as standard deviation). Melt temperature mean and standard deviations are shown in **Table 3**. for control and organism assays for the three test sites and

overall. Variability in the melt temperatures for the assays evaluated was within the expected range ( $\leq 0.5^{\circ}\text{C}$ ) for each assay at each site and overall, with a standard deviation of 0.2-0.3 $^{\circ}\text{C}$ .

**Table 3: Summary of T<sub>m</sub> ( $^{\circ}\text{C}$ ) Analyses for FilmArray Global Fever Special Pathogens Panel Assays**

| Analyte<br>(Isolate tested) | Assay           | Observed Tm (°C) |        |        |        |        |        |           |        |
|-----------------------------|-----------------|------------------|--------|--------|--------|--------|--------|-----------|--------|
|                             |                 | Site 1           |        | Site 2 |        | Site 3 |        | All Sites |        |
|                             |                 | Mean             | ±StDev | Mean   | ±StDev | Mean   | ±StDev | Mean      | ±StDev |
| CONTROLS                    |                 |                  |        |        |        |        |        |           |        |
| RNA Process Control         | yeast RNA       | 81.7             | ±0.2   | 81.6   | ±0.3   | 81.7   | ±0.2   | 81.6      | ±0.2   |
| PCR2 Control                | PCR2            | 75.5             | ±0.3   | 75.4   | ±0.3   | 75.6   | ±0.2   | 75.5      | ±0.3   |
| BACTERIA                    |                 |                  |        |        |        |        |        |           |        |
| Leptospira interrogans      | Lepto1          | 81.4             | ±0.2   | 81.3   | ±0.3   | 81.5   | ±0.2   | 81.4      | ±0.2   |
| VIRUSES                     |                 |                  |        |        |        |        |        |           |        |
| Dengue virus Type 2         | DENV2_1         | 80.1             | ±0.2   | 80.0   | ±0.3   | 80.1   | ±0.2   | 80.1      | ±0.2   |
| PROTOZOA                    |                 |                  |        |        |        |        |        |           |        |
| Plasmodium falciparum       | Plas spp.       | 79.0             | ±0.2   | 78.9   | ±0.2   | 79.0   | ±0.2   | 78.9      | ±0.2   |
|                             | Plas falciparum | 74.0             | ±0.2   | 74.0   | ±0.2   | 74.1   | ±0.2   | 74.0      | ±0.2   |
| Leishmania donovani         | Leish           | 85.4             | ±0.2   | 85.3   | ±0.2   | 85.4   | ±0.2   | 85.4      | ±0.2   |

Variability in the melt temperatures for the assays evaluated was within the expected range ( $\leq 0.5^{\circ}\text{C}$ ) for each assay at each site and overall, with a standard deviation of 0.2-0.3 $^{\circ}\text{C}$ .

Cumulatively, the results suggest that there are no significant differences between variables evaluated in the reproducibility study. Therefore, the reproducibility studies conducted for the BioFire Global Fever Special Pathogens Panel are acceptable.

Additional testing was also performed to demonstrate equivalent performance of the Global Fever Special Pathogens Panel on both the FilmArray 2.0 and FilmArray Torch instruments. Instead of performing the evaluation at three different sites, individual sites were simulated using different FilmArray Torch and different FilmArray 2.0 systems and two different operators for each of the simulated sites. Three contrived whole blood samples were prepared with different mixtures of six representative panel analytes, two bacteria, two viruses, and two protozoa. For each analyte, one sample was spiked at a Moderate Positive ( $3 \times \text{LoD}$ ) level, another sample at a Low Positive ( $1 \times \text{LoD}$ ) level, and the third sample was not spiked (negative). Six replicates of each sample were tested at each simulated site on five different days, providing a total of 90 replicate test results per sample per FilmArray system. On each test day, two operators per simulated site used three FilmArray 2.0 instruments and three FilmArray Torch modules; three Global Fever Special Pathogens Panel pouch reagent lots were rotated daily. Results of this study are summarized in Table 4 and Table 5 below.

**Table 4. Reproducibility of the Global Fever Special Pathogens Panel on FilmArray Torch and FilmArray 2.0 Instruments.**

| Analyte<br>(Source / ID)                                         |                                                        | Concentration Tested<br>(copies/mL) | Expected Result | Detection Rate (n/N)<br>% Agreement with Expected Result |                |                |                                              |                          |                |                                  |
|------------------------------------------------------------------|--------------------------------------------------------|-------------------------------------|-----------------|----------------------------------------------------------|----------------|----------------|----------------------------------------------|--------------------------|----------------|----------------------------------|
|                                                                  |                                                        |                                     |                 | FilmArray 2.0 Platform                                   |                |                |                                              | FilmArray Torch Platform |                |                                  |
|                                                                  |                                                        |                                     |                 | System 1                                                 | System 2       | System 3       | All FA 2.0 Systems<br>[95% CI]               | System 1                 | System 2       | All FA Torch Systems<br>[95% CI] |
| <i>Leptospira interrogans</i> serovar <i>icterohaemorrhagiae</i> |                                                        | 3×LoD (1.0E+03)                     | Detected        | 30/30<br>100%                                            | 30/30<br>100%  | 30/30<br>100%  | <b>90/90</b><br><b>100%</b><br>[95.9-100%]   | 30/30<br>100%            | 30/30<br>100%  | 30/30<br>100%                    |
|                                                                  |                                                        | 1×LoD (3.4E+02)                     | Detected        | 29/30<br>96.7%                                           | 29/30<br>96.7% | 28/30<br>93.3% | <b>86/90</b><br><b>95.6%</b><br>[89.1-98.3%] | 29/30<br>96.7%           | 28/30<br>93.3% | 28/30<br>93.3%                   |
|                                                                  |                                                        | Negative                            | Not Detected    | 30/30<br>100%                                            | 30/30<br>100%  | 30/30<br>100%  | <b>90/90</b><br><b>100%</b><br>[95.9-100%]   | 30/30<br>100%            | 30/30<br>100%  | 30/30<br>100%                    |
| <b>Dengue virus DENV-2</b><br>New Guinea C                       |                                                        | 3×LoD (1.0E+03)                     | Detected        | 30/30<br>100%                                            | 30/30<br>100%  | 30/30<br>100%  | <b>90/90</b><br><b>100%</b><br>[95.9-100%]   | 30/30<br>100%            | 30/30<br>100%  | 30/30<br>100%                    |
|                                                                  |                                                        | 1×LoD (3.4E+02)                     | Detected        | 30/30<br>100%                                            | 30/30<br>100%  | 30/30<br>100%  | <b>90/90</b><br><b>100%</b><br>[95.9-100%]   | 30/30<br>100%            | 29/30<br>96.7% | 30/30<br>100%                    |
|                                                                  |                                                        | Negative                            | Not Detected    | 30/30<br>100%                                            | 30/30<br>100%  | 30/30<br>100%  | <b>90/90</b><br><b>100%</b><br>[95.9-100%]   | 30/30<br>100%            | 30/30<br>100%  | 29/30 <sup>1</sup><br>96.7%      |
| <i>Leishmania donovani</i> 15 (MHOM/SD/62/1S)                    |                                                        | 3×LoD (1.0E+03)                     | Detected        | 30/30<br>100%                                            | 30/30<br>100%  | 30/30<br>100%  | <b>90/90</b><br><b>100%</b><br>[95.9-100%]   | 30/30<br>100%            | 30/30<br>100%  | 30/30<br>100%                    |
|                                                                  |                                                        | 1×LoD (3.4E+02)                     | Detected        | 30/30<br>100%                                            | 30/30<br>100%  | 30/30<br>100%  | <b>90/90</b><br><b>100%</b><br>[95.9-100%]   | 30/30<br>100%            | 30/30<br>100%  | 30/30<br>100%                    |
|                                                                  |                                                        | Negative                            | Not Detected    | 30/30<br>100%                                            | 30/30<br>100%  | 30/30<br>100%  | <b>90/90</b><br><b>100%</b><br>[95.9-100%]   | 30/30<br>100%            | 30/30<br>100%  | 30/30<br>100%                    |
| <i>Plasmodium falciparum</i> IPC 4884 (BEI / MRA-1238)           | <b>Plasmodium spp.</b><br><br><b>Detection Results</b> | 3×LoD (5.4E+02)                     | Detected        | 30/30<br>100%                                            | 30/30<br>100%  | 30/30<br>100%  | <b>90/90</b><br><b>100%</b><br>[95.9-100%]   | 30/30<br>100%            | 30/30<br>100%  | 30/30<br>100%                    |
|                                                                  |                                                        | 1×LoD (1.8E+02)                     | Detected        | 30/30<br>100%                                            | 30/30<br>100%  | 30/30<br>100%  | <b>90/90</b><br><b>100%</b><br>[95.9-100%]   | 30/30<br>100%            | 30/30<br>100%  | 30/30<br>100%                    |
|                                                                  |                                                        | Negative                            | Not Detected    | 30/30<br>100%                                            | 30/30<br>100%  | 30/30<br>100%  | <b>90/90</b><br><b>100%</b><br>[95.9-100%]   | 30/30<br>100%            | 30/30<br>100%  | 30/30<br>100%                    |

| Analyte<br>(Source / ID)               |                      | Concentrati<br>on Tested<br>(copies/mL) | Expected<br>Result | Detection Rate (n/N)<br>% Agreement with Expected Result |                |                |                                                  |                                    |                |                |                                                  |
|----------------------------------------|----------------------|-----------------------------------------|--------------------|----------------------------------------------------------|----------------|----------------|--------------------------------------------------|------------------------------------|----------------|----------------|--------------------------------------------------|
|                                        |                      |                                         |                    | FilmArray 2.0 Platform                                   |                |                |                                                  | FilmArray Torch Platform           |                |                |                                                  |
|                                        |                      |                                         |                    | System<br>1                                              | System<br>2    | System<br>3    | All FA 2.0<br>Systems<br>[95% CI]                | System<br>1                        | System<br>2    | System<br>3    | All FA<br>Torch<br>Systems<br>[95% CI]           |
| <i>Plasmodium<br/>falciparum</i>       |                      |                                         |                    |                                                          |                |                | [95.9-<br>100%]                                  |                                    |                |                | [95.9-<br>100%]                                  |
|                                        | Detection<br>Results | 3×LoD<br>(5.4E+02)                      | Detected           | 30/30<br>100%                                            | 30/30<br>100%  | 30/30<br>100%  | <b>90/90</b><br><b>100%</b><br>[95.9-<br>100%]   | 30/30<br>100%                      | 29/30<br>96.7% | 30/30<br>100%  | <b>89/90</b><br><b>98.9%</b><br>[94.0-<br>99.8%] |
|                                        |                      | 1×LoD<br>(1.8E+02)                      | Detected           | 30/30<br>100%                                            | 28/30<br>93.3% | 29/30<br>96.7% | <b>87/90</b><br><b>96.7%</b><br>[90.7-<br>98.9%] | 30/30<br>100%                      | 28/30<br>93.3% | 28/30<br>93.3% | <b>86/90</b><br><b>95.6%</b><br>[89.1-<br>98.3%] |
|                                        |                      | Negative                                | Not<br>Detected    | 30/30<br>100%                                            | 30/30<br>100%  | 30/30<br>100%  | <b>90/90</b><br><b>100%</b><br>[95.9-<br>100%]   | 30/30<br>100%                      | 30/30<br>100%  | 30/30<br>100%  | <b>90/90</b><br><b>100%</b><br>[95.9-<br>100%]   |
| Overall Agreement with Expected Result |                      |                                         |                    | 1343/1350<br>99.5%<br>[98.9-99.8%]                       |                |                |                                                  | 1338/1350<br>99.1%<br>[98.4-99.5%] |                |                |                                                  |

**Table 5. Summary of T<sub>m</sub> (°C) Analyses for FilmArray Global Fever Special Pathogens Panel Assays on FilmArray 2.0 and FilmArray Torch instruments**

| Analyte                | Assay     | FA Platform | Observed<br>Tm (°C) |        |      |                  |        |      |                  |        |      |         |        |      |
|------------------------|-----------|-------------|---------------------|--------|------|------------------|--------|------|------------------|--------|------|---------|--------|------|
|                        |           |             | Simulated Site 1    |        |      | Simulated Site 2 |        |      | Simulated Site 3 |        |      | Overall |        |      |
|                        |           |             | Mean                | ±StDev | CV   | Mean             | ±StDev | CV   | Mean             | ±StDev | CV   | Mean    | ±StDev | CV   |
| CONTROLS               |           |             |                     |        |      |                  |        |      |                  |        |      |         |        |      |
| RNA Process Control    | Yeast RNA | FA 2.0      | 82.0                | 0.3    | 0.4% | 82.1             | 0.3    | 0.4% | 82.1             | 0.3    | 0.4% | 82.1    | 0.3    | 0.4% |
|                        |           | Torch       | 82.4                | 0.2    | 0.2% | 82.3             | 0.2    | 0.2% | 82.3             | 0.2    | 0.2% | 82.3    | 0.2    | 0.2% |
| PCR2 Control           | PCR2      | FA 2.0      | 76.0                | 0.3    | 0.4% | 76.0             | 0.2    | 0.3% | 76.1             | 0.2    | 0.3% | 76.0    | 0.2    | 0.3% |
|                        |           | Torch       | 76.3                | 0.2    | 0.3% | 76.2             | 0.2    | 0.3% | 76.2             | 0.2    | 0.3% | 76.2    | 0.2    | 0.3% |
| BACTERIA               |           |             |                     |        |      |                  |        |      |                  |        |      |         |        |      |
| Leptospira interrogans | Lepto 1   | FA 2.0      | 81.7                | 0.3    | 0.4% | 81.7             | 0.3    | 0.4% | 81.8             | 0.2    | 0.2% | 81.8    | 0.3    | 0.4% |
|                        |           | Torch       | 82.0                | 0.2    | 0.2% | 81.9             | 0.2    | 0.2% | 81.9             | 0.2    | 0.2% | 82.0    | 0.2    | 0.2% |
| VIRUSES                |           |             |                     |        |      |                  |        |      |                  |        |      |         |        |      |
| Dengue virus Type 2    | DENV 2_1  | FA 2.0      | 80.3                | 0.3    | 0.4% | 80.3             | 0.2    | 0.2% | 80.4             | 0.2    | 0.2% | 80.3    | 0.2    | 0.2% |
|                        |           | Torch       | 80.6                | 0.2    | 0.2% | 80.5             | 0.2    | 0.2% | 80.5             | 0.2    | 0.2% | 80.5    | 0.3    | 0.4% |
| PROTOZOA               |           |             |                     |        |      |                  |        |      |                  |        |      |         |        |      |
|                        | Leish     | FA 2.0      | 85.6                | 0.3    | 0.4% | 85.6             | 0.2    | 0.2% | 85.7             | 0.2    | 0.2% | 85.6    | 0.2    | 0.2% |

|                              |                 |        |      |     |      |      |     |      |      |     |      |      |     |      |
|------------------------------|-----------------|--------|------|-----|------|------|-----|------|------|-----|------|------|-----|------|
| <i>Leishmania donovani</i>   |                 | Torch  | 86.1 | 0.2 | 0.2% | 85.9 | 0.2 | 0.2% | 85.9 | 0.2 | 0.2% | 86.0 | 0.2 | 0.2% |
| <i>Plasmodium falciparum</i> | Plas spp.       | FA 2.0 | 79.1 | 0.3 | 0.4% | 79.2 | 0.3 | 0.4% | 79.3 | 0.2 | 0.3% | 79.2 | 0.2 | 0.3% |
|                              |                 | Torch  | 79.5 | 0.2 | 0.3% | 79.4 | 0.2 | 0.3% | 79.4 | 0.2 | 0.3% | 79.4 | 0.2 | 0.3% |
|                              | Plas falciparum | FA 2.0 | 74.2 | 0.3 | 0.4% | 74.3 | 0.2 | 0.3% | 74.3 | 0.2 | 0.3% | 74.3 | 0.2 | 0.3% |
|                              |                 | Torch  | 74.6 | 0.2 | 0.3% | 74.5 | 0.2 | 0.3% | 74.5 | 0.2 | 0.3% | 74.5 | 0.2 | 0.3% |

Cumulatively, these data demonstrate that no performance difference is expected for the Global Fever Special Pathogens Panel between the FilmArray 2.0 and FilmArray Torch instrument systems.

Reproducibility of the BioFire SHIELD Control Kit for the BioFire Global Fever Special Pathogens Panel was also evaluated on both the FilmArray 2.0 and FilmArray Torch Systems. Briefly, the control material was assessed for reproducibility over three different instrument systems and three independent reagent lots. Testing took place over five days with evaluation of the FilmArray 2.0 instrument runs occurring across three separate sites and evaluation of FilmArray Torch instrument runs occurring at BioFire across three simulated sites. Results from these evaluations are summarized below in Table 6 and Table 7.

**Table 6. Reproducibility Test Results of the BioFire SHIELD Control Kit for the Global Fever Special Pathogens Panel on the FilmArray 2.0 System**

| External Control Type                  | Expected Result     | Observed/Expected<br>(Percent Agreement) |                               |                               |                                        |
|----------------------------------------|---------------------|------------------------------------------|-------------------------------|-------------------------------|----------------------------------------|
|                                        |                     | Site 1                                   | Site 2                        | Site 3                        | All Sites<br>[95% Confidence Interval] |
| Positive                               | Passed <sup>1</sup> | 42/45<br>(93.3%)                         | 45/45<br>(100%)               | 45/45<br>(100%)               | 132/135<br>(97.8%)<br>[93.7-100%]      |
| Negative                               | Passed <sup>2</sup> | 45/45<br>(100%)                          | 44/45 <sup>3</sup><br>(97.8%) | 44/45 <sup>4</sup><br>(97.8%) | 133/135<br>(98.5%)<br>[94.8-99.6%]     |
| Overall Agreement with Expected Result |                     | 265/270 (98.1%)<br>[95.7-99.2%]          |                               |                               |                                        |

<sup>1</sup>All GF Special Pathogens Panel assays have a positive amplicon melt in the ECM melt range.

<sup>2</sup>All GF special Pathogens Panel assays have no melt in both the ECM melt range and in the organism/virus melt range.

<sup>3</sup>Unexpected detection of pathogen amplicon for Leish assay.

<sup>4</sup>Unexpected detection of pathogen amplicon for DENV 2\_1 assay.

Three Positive External Controls failed; one failed due a single assay being called negative, and the other two showed no amplification of ECM for any of the Global Fever Special Pathogens Panel assays.

**Table 7. Reproducibility Test Results of the BioFire SHIELD Control Kit for the Global Fever Special Pathogens Panel on the FilmArray Torch System**

| External Control Type                  | Expected Result     | Observed/Expected<br>(Percent Agreement) |                  |                 |                                      |
|----------------------------------------|---------------------|------------------------------------------|------------------|-----------------|--------------------------------------|
|                                        |                     | System 1                                 | System 2         | System 3        | Overall<br>[95% Confidence Interval] |
| Positive                               | Passed <sup>1</sup> | 43/45<br>(95.6%)                         | 43/45<br>(95.6%) | 44/45<br>(100%) | 130/135<br>(96.3%)<br>[91.6-98.4%]   |
| Negative                               | Passed <sup>2</sup> | 43/45 <sup>3</sup><br>(95.6%)            | 45/45<br>(100%)  | 45/45<br>(100%) | 133/135<br>(98.5%)<br>[94.8-99.6%]   |
| Overall Agreement with Expected Result |                     | 263/270 (97.4%)<br>[94.7-98.7%]          |                  |                 |                                      |

<sup>1</sup>All GF Special Pathogens Panel assays have a positive amplicon melt in the ECM melt range.

<sup>2</sup>All GF Special Pathogens Panel assays have no melt in both the ECM melt range and in the organism/virus melt range.

<sup>3</sup>One run had unexpected positive results for Ebolavirus and West Nile virus assays and another run had unexpected positive results for Dengue, Ebolavirus, and *P. falciparum* assays

2. Linearity:

Not applicable.

3. Analytical Specificity/Interference:

*Inclusivity*

The analytical reactivity (inclusivity) of the BioFire Global Fever Special Pathogens Panel was evaluated with a collection of isolates to represent relevant species, subspecies, or serotypes. Testing was performed by evaluating three replicates. If there was one undetected result, two additional replicates were tested and if both replicates were detected the isolate was considered inclusive. Most isolates were detected by the FilmArray Global Fever Panel at spiked concentrations within  $3 \times \text{LoD}$  of testing either inactivated or live organisms (based on molecular quantification of nucleic acids for each isolate) in whole blood. Several isolates with reduced assay reactivity are described in more detail below.

When possible, in silico analysis of sequence data was used to make predictions of assay reactivity for less common strains or serotypes that were not tested. Additional inclusivity testing of live organisms for BSL3/4 organisms was also performed the results from this testing have been incorporated into the table below.

Table 8 includes a summary of FilmArray Global Fever Panel reactivity based on empirical data.

**Table 8. Summary of FilmArray Global Fever Panel Analytical Reactivity (Inclusivity)**

| FilmArray Global Fever Panel Analyte | # Isolates Detected / Tested | Isolates Tested          |                                | Concentration Detected <sup>1</sup> | x LoD     | FilmArray Global Fever Panel Result (Replicates Detected/Tested) |
|--------------------------------------|------------------------------|--------------------------|--------------------------------|-------------------------------------|-----------|------------------------------------------------------------------|
| BACTERIA                             |                              |                          |                                |                                     |           |                                                                  |
| <i>Bacillus anthracis</i>            | 4/4                          | Ames35                   |                                | 4.2E+01 copies/mL                   | 1 x LoD   | Detected (19/20)                                                 |
|                                      |                              | Sterne 34Fs              |                                | 1.3E+02 copies/mL                   | 2.5 x LoD | Detected (3/3)                                                   |
|                                      |                              | UM23                     |                                |                                     |           |                                                                  |
|                                      |                              | Weybridge                |                                |                                     |           |                                                                  |
|                                      | 10/12                        | Ames (Live)              |                                | 6.4E+01 copies/mL                   | 1 x LoD   | Detected (4/4)                                                   |
|                                      |                              | 108 (Live)               |                                | 1.9E+02 copies/mL                   | 3 x LoD   | Detected (3/3)                                                   |
|                                      |                              | 2002013094 (Live)        |                                |                                     |           |                                                                  |
|                                      |                              | Canadian Bison (Live)    |                                |                                     |           |                                                                  |
|                                      |                              | K3 (Live)                |                                |                                     |           |                                                                  |
|                                      |                              | Ohio ACB (Live)          |                                |                                     |           |                                                                  |
|                                      |                              | PAK-1 (Live)             |                                |                                     |           |                                                                  |
|                                      |                              | RA3 (Live)               |                                |                                     |           |                                                                  |
|                                      |                              | SK-102 (Live)            |                                | 6.4E+02 copies/mL                   | 10 x LoD  | Detected (4/5)                                                   |
|                                      |                              | South Africa (Live)      |                                | 1.9E+02 copies/mL                   | 3 x LoD   | Detected (3/3)                                                   |
|                                      |                              | Sterne (Live)            |                                |                                     |           |                                                                  |
|                                      |                              | Turkey #32 (Live)        |                                |                                     |           |                                                                  |
|                                      |                              | Vollum 1B (Live)         |                                | 6.4E+02 copies/mL                   | 10 x LoD  | Detected (3/3)                                                   |
| <i>Francisella tularensis</i>        | 5/5                          | Subspecies               | Strain                         |                                     |           |                                                                  |
|                                      |                              | <i>F. tularensis</i>     | SCHU S4                        | 1.2E+03 copies/mL                   | 1 x LoD   | Detected (20/20)                                                 |
|                                      |                              | <i>F. holarctica</i>     | LVSR                           | 3.6E+03 copies/mL                   | 3 x LoD   | Detected (3/3)                                                   |
|                                      |                              |                          | Type B LVS (CDC)               | 3.6E+03 copies/mL                   | 3 x LoD   | Detected (3/3)                                                   |
|                                      |                              | <i>F. novicida</i>       | KM14S                          | 3.6E+03 copies/mL                   | 3 x LoD   | Detected (3/3)                                                   |
|                                      |                              |                          | CG62                           | 3.6E+03 copies/mL                   | 3 x LoD   |                                                                  |
|                                      | 9/9                          | <i>F. tularensis</i>     | Schu4 (Live)                   | 1.2E+01 copies/mL                   | 1 x LoD   | Detected (4/4)                                                   |
|                                      |                              |                          | Scherm (Live)                  | 3.6E+01 copies/mL                   | 3 x LoD   | Detected (3/3)                                                   |
|                                      |                              |                          | WY96 (Live)                    |                                     |           |                                                                  |
|                                      |                              | <i>F. holarctica</i>     | Holarctica LVS (Live)          |                                     |           |                                                                  |
|                                      |                              |                          | Holarctica (Live)              |                                     |           |                                                                  |
|                                      |                              |                          | Holarctica 425 (live)          |                                     |           |                                                                  |
|                                      |                              |                          | HD, DB082106G (Live)           | 2.3E+01 copies/mL                   | 1.9 x LoD |                                                                  |
|                                      |                              | <i>F. novicida</i>       | VT68 (Live)                    | 3.6E+01 copies/mL                   | 3 x LoD   |                                                                  |
| F6168 (Live)                         |                              |                          |                                |                                     |           |                                                                  |
| U112, GA993550 (Live)                |                              |                          |                                |                                     |           |                                                                  |
| <i>Leptospira</i> spp.               | 19/19                        | Species                  | Strain                         |                                     |           |                                                                  |
|                                      |                              | <i>L.interrogans</i>     | Serovar (Budapest)             | 3.4E+02 copies/mL                   | 1 x LoD   | Detected (20/20)                                                 |
|                                      |                              |                          | HAI0156 (Copenhageni)          | 1.2E+03 copies/mL                   | 3.5 x LoD | Detected (3/3)                                                   |
|                                      |                              |                          | L495 (Manilae)                 |                                     |           |                                                                  |
|                                      |                              | <i>L. alexanderi</i>     | L60 (Manhao 3)                 | 1.0E+03 copies/mL                   | 3 x LoD   |                                                                  |
|                                      |                              | <i>L. alstonii</i>       | Sichuan 79601                  | 1.2E+03 copies/mL                   | 3.5 x LoD | Detected (4/5)                                                   |
|                                      |                              | <i>L. borgpetersenii</i> | Castellon 3 (Castellonis)      |                                     |           | Detected (3/3)                                                   |
|                                      |                              |                          | Veldrat Bataviae 46 (Javanica) | 1.0E+03 copies/mL                   | 3 x LoD   |                                                                  |
| <i>L. kirschneri</i>                 | 200701401 (Bogvere)          | 1.2E+03 copies/mL        | 3.5 x LoD                      |                                     |           |                                                                  |

| FilmArray Global Fever Panel Analyte | # Isolates Detected / Tested | Isolates Tested            |                        | Concentration Detected <sup>1</sup> | x LoD     | FilmArray Global Fever Panel Result (Replicates Detected/Tested) |  |  |
|--------------------------------------|------------------------------|----------------------------|------------------------|-------------------------------------|-----------|------------------------------------------------------------------|--|--|
|                                      |                              |                            | 3522 C (Cynopteri)     | 5.3E+02 copies/mL                   | 1.6 x LoD |                                                                  |  |  |
|                                      |                              | L. kmetyi                  | Bejo-Iso9T (Malaysia)  | 1.2E+03 copies/mL                   | 3.5 x LoD |                                                                  |  |  |
|                                      |                              | L. mayottensis             | 200901116              |                                     |           |                                                                  |  |  |
|                                      |                              | L. noguchii                | CZ 214T (Panama)       |                                     |           |                                                                  |  |  |
|                                      |                              | L. santarosai              | LT 821 (Shermani)      | 8.7E+02 copies/mL                   | 2.6 x LoD |                                                                  |  |  |
|                                      |                              | L. weilii                  | 6712                   | 1.2E+03 copies/mL                   | 3.5 x LoD |                                                                  |  |  |
|                                      |                              |                            | 94-79970/3 Topaz       |                                     |           |                                                                  |  |  |
|                                      |                              |                            | A 102 (Mengrun)        |                                     |           |                                                                  |  |  |
|                                      |                              |                            | Celledoni 20160426     |                                     |           |                                                                  |  |  |
|                                      |                              |                            | H 27 (Hekooou)         |                                     |           |                                                                  |  |  |
|                                      |                              |                            | LT89-68 (Vughia)       |                                     |           |                                                                  |  |  |
| Yersinia pestis                      | 3/3                          | A1122 (biovar Orientalis)  |                        | 1.3E+02 copies/mL                   | 1 x LoD   | Detected (20/20)                                                 |  |  |
|                                      |                              | PY-013 (biovar Orientalis) |                        | 3.9E+02 copies/mL                   | 3 x LoD   | Detected (3/3)                                                   |  |  |
|                                      |                              | PH 80/63 (biovar Antiqua)  |                        | 3.9E+02 copies/mL                   | 3 x LoD   | Detected (3/3)                                                   |  |  |
|                                      | 12/12                        | Biovar                     | Strain                 |                                     |           |                                                                  |  |  |
|                                      |                              | Orientalis                 | CO92 (Live)            | 1.5E+02 copies/mL                   | 1 x LoD   | Detected (8/8)                                                   |  |  |
|                                      |                              |                            | A1122 (Live)           | 4.5E+02 copies/mL                   | 3 x LoD   | Detected (3/3)                                                   |  |  |
|                                      |                              |                            | Dodson (Live)          | 3.6E+02 CFU/mL                      | 3 x LoD   | Detected (3/3)                                                   |  |  |
|                                      |                              |                            | Java 9 (Live)          | 4.5E+02 copies/mL                   | 3 x LoD   | Detected (3/3)                                                   |  |  |
|                                      |                              |                            | PBM19 (Live)           |                                     |           |                                                                  |  |  |
|                                      |                              |                            | Shasta (Live)          |                                     |           |                                                                  |  |  |
|                                      |                              | Angola (Live)              |                        |                                     |           |                                                                  |  |  |
|                                      |                              | Antigua                    | Antigua (Live)         | 3.6E+02 copies/mL                   | 3 x LoD   | Detected (3/3)                                                   |  |  |
|                                      |                              |                            | Nairobi (Live)         |                                     |           |                                                                  |  |  |
|                                      |                              |                            | Pestoides F (Live)     | 3.6E+02 CFU/mL                      |           |                                                                  |  |  |
|                                      |                              |                            | Medievalis             | Harbin 35 (Live)                    |           |                                                                  |  |  |
|                                      |                              | KIM5 (Live)                |                        | 6.4E+02 copies/mL                   | 4.2 x LoD |                                                                  |  |  |
|                                      |                              | Nicholisk 41 (Live)        |                        | 4.5E+02 copies/mL                   | 3 x LoD   |                                                                  |  |  |
|                                      |                              | VIRUSES                    |                        |                                     |           |                                                                  |  |  |
|                                      |                              | Chikungunya Virus          | 3/3                    | Strain                              |           |                                                                  |  |  |
| R80422                               |                              |                            |                        | 5.5E+02 copies/mL                   | 1 x LoD   | Detected (20/20)                                                 |  |  |
| DHS4263                              |                              |                            |                        | 1.7E+03 copies/mL                   | 3 x LoD   | Detected (3/3)                                                   |  |  |
| St. Martin 2013                      |                              |                            |                        | 1.4E+03 copies/mL                   | 2.5 x LoD |                                                                  |  |  |
| Crimean-Congo Hemorrhagic Virus      | 1/1                          | Nigeria/IbAr10200          |                        | 6.4E+00 copies/mL                   | 1 x LoD   | Detected (20/20)                                                 |  |  |
| Dengue virus                         | 27/28                        | Serotype                   | Strain                 |                                     |           |                                                                  |  |  |
|                                      |                              | Serotype 1                 | Hawaii                 | 2.2E+02 copies/mL                   | 1 x LoD   | Detected (19/20)                                                 |  |  |
|                                      |                              |                            | Strain 12150           | 6.6E+02 copies/mL                   | 3 x LoD   | Detected (3/3)                                                   |  |  |
|                                      |                              |                            | 228690                 |                                     |           |                                                                  |  |  |
|                                      |                              |                            | 276RK1                 |                                     |           |                                                                  |  |  |
|                                      |                              |                            | BC89/94                |                                     |           |                                                                  |  |  |
|                                      |                              |                            | SL-6-6-04              |                                     |           |                                                                  |  |  |
|                                      |                              |                            | UIS 1162               |                                     |           |                                                                  |  |  |
|                                      |                              |                            | VN/BID-V1792/2007      |                                     |           |                                                                  |  |  |
|                                      |                              | Serotype 2                 | New Guinea C (DENV2 1) | 3.4E+02 copies/mL                   | 1 x LoD   | Detected (20/20)                                                 |  |  |
|                                      |                              |                            | DakArA1247 (DENV2 2)   | 2.7E+03 copies/mL                   | 1 x LoD   |                                                                  |  |  |
|                                      |                              |                            | 1349                   | 1.1E+03 copies/mL                   | 3.2 x LoD | Detected (3/3)                                                   |  |  |
|                                      |                              |                            | 429557                 |                                     |           |                                                                  |  |  |

| FilmArray Global Fever Panel Analyte | # Isolates Detected / Tested | Isolates Tested          |                    | Concentration Detected <sup>1</sup> | x LoD         | FilmArray Global Fever Panel Result (Replicates Detected/Tested) |
|--------------------------------------|------------------------------|--------------------------|--------------------|-------------------------------------|---------------|------------------------------------------------------------------|
|                                      |                              |                          | ArA6894            | 1.1E+03 copies/mL                   | 0.4 x LoD     | Detected (1/1)                                                   |
|                                      |                              |                          | BC102/94           | 9.4E+02 copies/mL                   | 2.8 x LoD     |                                                                  |
|                                      |                              |                          | DKA 811            | 2.4E+06 copies/mL                   | ~10,000 x LoD |                                                                  |
|                                      |                              |                          | VN/BID-V1002/2006  | 1.1E+03 copies/mL                   | 3.2 x LoD     |                                                                  |
|                                      |                              | Serotype 3               | H87                | 1.3E+02 copies/mL                   | 1 x LoD       | Detected (20/20)                                                 |
|                                      |                              |                          | 271242             | 3.9E+02 copies/mL                   | 3 x LoD       | Detected (3/3)                                                   |
|                                      |                              |                          | BC188/97           | 1.6E+04 copies/mL                   | 123 x LoD     |                                                                  |
|                                      |                              |                          | C0360/94           | 3.9E+02 copies/mL                   | 3 x LoD       |                                                                  |
|                                      |                              |                          | VN/BID-V1329/2006  |                                     |               |                                                                  |
|                                      |                              | Serotype 4               | H241               | 6.4E+01 copies/mL                   | 1 x LoD       | Detected (20/20)                                                 |
|                                      |                              |                          | 703                | 1.9E+02 copies/mL                   | 3 x LoD       | Detected (3/3)                                                   |
|                                      |                              |                          | BC13/97            |                                     |               |                                                                  |
|                                      |                              |                          | BC287/97           |                                     |               |                                                                  |
|                                      |                              |                          | BC258/97           |                                     |               |                                                                  |
|                                      |                              |                          | D85-019            | 7.6E+03 copies/mL                   | 119 x LoD     |                                                                  |
|                                      |                              | PR 06-65-740             | 2.2E+02 copies/mL  | 3.4 x LoD                           |               |                                                                  |
| Ebola <span>virus</span> spp.        | 6/6                          | Bundibugyo virus Uganada |                    | 7.0E+04 copies/mL                   | 1 x LoD       | Detected (20/20)                                                 |
|                                      |                              | Tai Forest virus         |                    | 8.3E+03 copies/mL                   | 1 x LoD       |                                                                  |
|                                      |                              | Reston virus             |                    | 2.7E+04 copies/mL                   | 1 x LoD       |                                                                  |
|                                      |                              | Sudan virus              |                    | 1.1E+04 copies/mL                   | 1 x LoD       |                                                                  |
|                                      |                              | Zaire                    | Gueckedou          | 1.1E+02 copies/mL                   | 1 x LoD       |                                                                  |
|                                      | 4/4                          | Zaire                    | Mayinga            | 1.1E+04 copies/mL                   | 110 x LoD     | Detected (3/3)                                                   |
|                                      |                              |                          | Makona (Live)      | 1.1E+03 copies/mL                   | 1 x LoD       |                                                                  |
|                                      |                              |                          | Mayinga '76 (Live) | 2.1E+03 copies/mL                   | 2 x LoD       |                                                                  |
|                                      |                              |                          | Gabon (Live)       | 3.2E+03 copies/mL                   | 2.9 x LoD     |                                                                  |
|                                      |                              |                          | Kikwit '95 (Live)  | 3.1E+03 copies/mL                   | 2.8 x LoD     |                                                                  |
| Luebo (Live)                         |                              |                          | 3.1E+03 copies/mL  | 2.8 x LoD                           |               |                                                                  |
| Lassa virus                          | 1/1                          | Josiah                   |                    | 5.6E+04 copies/mL                   | 1 x LoD       | Detected (20/20)                                                 |
|                                      | 2/2                          | Josiah (Live)            |                    | 2.4E+01 PFU/mL                      | 1 x LoD       | Detected (4/4)                                                   |
|                                      |                              | Macenta (Live)           |                    | 7.2E+01 PFU/mL                      | 3 x LoD       | Detected (3/3)                                                   |
|                                      |                              | Pinneo (Live)            |                    | 7.2E+01 PFU/mL                      | 3 x LoD       |                                                                  |
| Marburg <span>virus</span>           | 3/3                          | Musoke                   |                    | 5.0E+02 copies/mL                   | 1 x LoD       | Detected (20/20)                                                 |
|                                      |                              | Voegel                   |                    | 1.5E+03 copies/mL                   | 3 x LoD       | Detected (4/5)                                                   |
|                                      |                              | Ravn virus, Kenya        |                    | 2.6E+02 copies/mL                   | 1 x LoD       | Detected (20/20)                                                 |
|                                      | 2/2                          | Ravn (Live)              |                    | 2.6E+02 copies/mL                   | 1 x LoD       | Detected (4/4)                                                   |
|                                      |                              | Ci67 (Live)              |                    | 5.0E+02 copies/mL                   | 1 x LoD       | Detected (4/4)                                                   |
|                                      |                              | Angola (Live)            |                    | 1.5E+03 copies/mL                   | 3 x LoD       | Detected (3/3)                                                   |
|                                      |                              | Musoke (Live)            |                    | 7.8E+02 copies/mL                   | 1.5 x LoD     |                                                                  |
| West Nile virus                      | 5/5                          | B-956 Uganda             |                    | 2.3E+04 copies/mL                   | 1 x LoD       | Detected (20/20)                                                 |
|                                      |                              | B-956 (lineage 2)        |                    | 6.9E+04 copies/mL                   | 3 x LoD       | Detected (3/3)                                                   |
|                                      |                              | NY 2001-6263 (lineage 1) |                    | 1.1E+03 copies/mL                   | 1 x LoD       | Detected (20/20)                                                 |
|                                      |                              | Bird 114 (lineage 1)     |                    | 3.3E+03 copies/mL                   | 3 x LoD       | Detected (3/3)                                                   |
|                                      |                              | 1986 (lineage 1)         |                    | 2.2E+03 copies/mL                   | 2 x LoD       |                                                                  |
|                                      | 1/1                          | Bz NY99 (Live)           |                    | 1.6E+02 copies/mL                   | 1 x LoD       | Detected (4/4)                                                   |
|                                      |                              | Eg101 (Live)             |                    | 4.8E+02 copies/mL                   | 3 x LoD       | Detected (3/3)                                                   |
| Yellow Fever virus                   | 1/1                          | 17D                      |                    | 1.2E+02 copies/mL                   | 1 x LoD       | Detected (20/20)                                                 |
|                                      | 4/4                          | Asibi (Live)             |                    | 1.2E+01 copies/mL                   | 1 x LoD       | Detected (3/3)                                                   |
|                                      |                              | SVM 3-18-09 (Live)       |                    | 1.2E+04 copies/mL                   | 1000 x LoD    |                                                                  |
|                                      |                              | CAREC M2-09 (Live)       |                    | 1.2E+03 copies/mL                   | 100 x LoD     |                                                                  |
|                                      |                              | INHRR 7a-05 (Live)       |                    | 1.2E+03 copies/mL                   | 100 x LoD     | Detected (4/5)                                                   |
|                                      |                              | INHRR 10a-10 (Live)      |                    | 1.2E+03 copies/mL                   | 100 x LoD     |                                                                  |
| PROTOZOA                             |                              |                          |                    |                                     |               |                                                                  |
| Leishmania                           | 4/4                          | L. donovani 9515         |                    | 1.0E+01 copies/mL                   | 1 x LoD       | Detected (20/20)                                                 |

| FilmArray Global Fever Panel Analyte | # Isolates Detected / Tested | Isolates Tested            |                    | Concentration Detected <sup>1</sup> | x LoD     | FilmArray Global Fever Panel Result (Replicates Detected/Tested) |
|--------------------------------------|------------------------------|----------------------------|--------------------|-------------------------------------|-----------|------------------------------------------------------------------|
| spp.                                 |                              | <i>L. donovani</i> , 1S    |                    | 3.4E+01 copies/mL                   | 3.4 x LoD | Detected (3/3)                                                   |
|                                      |                              | <i>L. donovani</i> chagasi |                    | 3.0E+01 copies/mL                   | 3 x LoD   | Detected (4/5)                                                   |
|                                      |                              | <i>L. infantum</i>         |                    | 1.0E+02 copies/mL                   | 10 x LoD  | Detected (3/3)                                                   |
| Plasmodium spp.                      | 10/10                        | Species                    | Strain             |                                     |           |                                                                  |
|                                      |                              | <i>falciparum</i>          | IPC 4884           | 1.8E+02 copies/mL                   | 1 x LoD   | Detected (20/20)                                                 |
|                                      |                              |                            | SenTh021.09        | 3.0E+02 copies/mL                   | 1.7 x LoD | Detected (5/5)                                                   |
|                                      |                              |                            | St. Lucia          |                                     |           | Detected (3/3)                                                   |
|                                      |                              |                            | Tanzania, 02000708 |                                     |           |                                                                  |
|                                      |                              | <i>vivax</i>               | Chesson            | 1.5E+02 copies/mL                   | 1 x LOD   | Detected (20/20)                                                 |
|                                      |                              |                            | Panama             | 3.1E+02 copies/mL                   | 2.1 x LOD | Detected (3/3)                                                   |
|                                      |                              | <i>ovale</i>               | Wallikeri          | 2.4E+02 copies/mL                   | 1 x LOD   | Detected (20/20)                                                 |
|                                      |                              |                            | Curtisi            | 7.2E+02 copies/mL                   | 3 x LoD   | Detected (3/3)                                                   |
|                                      |                              | <i>knowlesi</i>            | Strain H           | 2.4E+01 copies/mL                   | 1 x LOD   | Detected (20/20)                                                 |
|                                      |                              | <i>malariae</i>            | Clinical specimen  | 1.9E+02 copies/mL                   | 1 x LOD   |                                                                  |
| <i>Plasmodium falciparum</i>         | 4/4                          | <i>falciparum</i>          | IPC4884            | 1.8E+02 copies/mL                   | 1 x LoD   | Detected (3/3)                                                   |
|                                      |                              |                            | SenTh021.09        | 1.8E+03 copies/mL                   | 10 x LoD  |                                                                  |
|                                      |                              |                            | St. Lucia          | 3.0E+02 copies/mL                   | 1.7 x LoD | Detected (4/5)                                                   |
|                                      |                              |                            | Tanzania, 02000708 |                                     |           | Detected (3/3)                                                   |
| <i>Plasmodium vivax/ovale</i>        | 4/4                          | <i>vivax</i>               | Chesson            | 1.5E+02 copies/mL                   | 1 x LoD   | Detected (20/20)                                                 |
|                                      |                              |                            | Panama             | 3.1E+02 copies/mL                   | 2 x LoD   | Detected (3/3)                                                   |
|                                      |                              | <i>ovale</i>               | Wallikeri          | 2.4E+02 copies/mL                   | 1 x LoD   | Detected (20/20)                                                 |
|                                      |                              |                            | Curtisi            | 7.2E+02 copies/mL                   | 3 x LoD   | Detected (3/3)                                                   |

Organisms which exhibited reduced or no assay reactivity are described specifically in the table below.

| Observed Result                 | Detection Level | Analyte                                   | Serotype/Strain/Isolate                   |
|---------------------------------|-----------------|-------------------------------------------|-------------------------------------------|
| Detected (may be underreported) | 10 x LoD        | <i>Bacillus anthracis</i>                 | SK-102 (Live)                             |
|                                 | 10 x LoD        | <i>Bacillus anthracis</i>                 | Vollum 1B (Live)                          |
|                                 | 10 x LoD        | <i>Leishmania infantum</i>                |                                           |
|                                 | 10 x LoD        | <i>Leishmania braziliensis</i>            | Vianna                                    |
|                                 | 10 x LoD        | <i>Plasmodium falciparum</i> <sup>1</sup> | SenTh021.09                               |
|                                 | ~100 x LoD      | Dengue virus <sup>1</sup>                 | Serotype 3 BC188/97                       |
|                                 |                 |                                           | Serotype 4 D85-019                        |
|                                 | 110 x LoD       | Ebolavirus                                | Zaire, Mayinga (Inactivated) <sup>3</sup> |
|                                 | 100 x LoD       | Yellow Fever Virus                        | CAREC M2-09 <sup>4</sup>                  |
|                                 |                 |                                           | INHRR 7a-05 <sup>4</sup>                  |
|                                 |                 |                                           | INHRR 10a-10 <sup>4</sup>                 |
|                                 | 1000 x LoD      |                                           | SVM 3-18-09 <sup>4</sup>                  |
| Not Detected                    | -               | Dengue virus <sup>2</sup>                 | Serotype 2 DKA 811                        |

<sup>1</sup>The reason for the observed reduced reactivity could not be identified based on *in silico* sequence analysis. Sequences for these strains were not available in public databases.

<sup>2</sup>*In silico* analysis predicted reduced sensitivity or missed detection of this isolate due to sequence variation. Wet testing of this rare sylvatic strain at 10,000 x LoD confirmed detection was significantly impaired.

<sup>3</sup>Results of live testing did not show reduced sensitivity for the detection of *Zaire ebolavirus* Mayinga, and therefore the observed reduced sensitivity for inactivated Zaire ebolavirus Mayinga may be the result of damage to nucleic acid due to inactivation process.

<sup>4</sup>YFV strains SVM 3-18-09, CAREC M2-09, NHRR 7a-05, and INHRR 10a-10 were isolated from neighboring regions (Trinidad and Venezuela). *In silico* analyses indicate these strains are closely related and do not represent a broad diversity of sequences. Available sequences for these strains do not contain the full target region of the assay, therefore the reduced sensitivity could not be fully investigated.

### Microbial Interference Studies

Potentially interfering microorganisms were evaluated for their effect on FilmArray Global Fever Special Pathogens Panel performance. To evaluate the potential for interference, FilmArray Global Fever Special Pathogens Panel test results from a control blood sample containing representative panel analytes (*Leptospira interrogans*, *P. falciparum*, dengue virus type 3) at concentrations near 3×LoD were compared to results from a sample with the same composition plus the potentially interfering microorganism, as well as a negative sample (no analytes) containing only the potentially interfering microorganism. Each potentially competing microorganism was tested at the highest concentration possible (1:10 dilution of the stock). The samples containing the potentially interfering microorganism were evaluated for their effects on the BioFire Global Fever Special Pathogens Panel internal control assays and analyte detection. Reproducible internal control failures or loss of analyte detection associated with the presence of a particular potentially interfering microorganism would be recognized as interference by that microorganism.

**Table 9. Organisms Evaluated for Potential Microbial Interference**

| Microorganisms                         | Concentration Tested                       | Results                  |
|----------------------------------------|--------------------------------------------|--------------------------|
| <i>Corynebacterium diphtheriae</i>     | 1:10 dilution of stock                     | No interference observed |
| <i>Staphylococcus epidermidis</i>      | 3.8E+06 CFU/mL                             | No interference observed |
| <i>Escherichia coli</i>                | 1:10 dilution of stock                     | No interference observed |
| <i>Klebsiella pneumoniae</i>           | 5.5E+04 CFU/mL                             | No interference observed |
| <i>Haemophilus influenzae</i>          | 1.0E+08 CFU/mL                             | No interference observed |
| Herpes Simplex virus                   | 1.2E+05 TCID <sub>50</sub> /mL             | No interference observed |
| Epstein-Barr virus                     | 3.3E+07 copies/mL                          | No interference observed |
| Cytomegalovirus                        | 1:10 dilution of stock                     | No interference observed |
| Human Immunodeficiency virus (HIV) 1/2 | HIV-1: 1.3E+05 U/mL<br>HIV-2: 2.2E+05 U/mL | No interference observed |
| <i>Plasmodium vivax</i>                | 1.5E+06 copies/mL                          | No interference observed |

None of the ten microorganisms tested showed interference with the pouch controls or specific Global Fever Panel assay targets.

### Analytical specificity/Cross-reactivity

The potential for non-specific amplification and detection by the FilmArray Global Fever Panel assays (cross-reactivity) was evaluated by testing high concentrations of on-panel (identified by the FilmArray Global Fever Panel assays) and off-panel (not intended to be identified by the FilmArray Global Fever Panel assays) organisms or purified nucleic acids, as well as by *in silico* analysis. All on-panel organisms were tested live at high concentration (> 10<sup>6</sup> copies/mL). As can be seen in Table 10 below, testing of *P. knowlesi* demonstrated cross-reactivity with the *P. vivax/ovale* assay at concentrations above 2.2E+03 copies/mL.

**Table 10. BioFireGlobal Fever Special Pathogens Panel Results for On-Panel Organism Testing Assessing Potential Cross-reactivity**

| Pathogen                                               |                        | Results                                                                                        |
|--------------------------------------------------------|------------------------|------------------------------------------------------------------------------------------------|
| <i>Bacillus anthracis</i>                              |                        | As expected (only <i>B. anthracis</i> Detected)                                                |
| <i>Francisella tularensis</i> subsp. <i>tularensis</i> |                        | As expected (onl <i>F. tularensis</i> Detected)                                                |
| <i>Leptospira interrogans</i> (Schu S4)                |                        | As expected (only <i>Leptospira spp.</i> Detected)                                             |
| <i>Yersinia Pestis</i>                                 |                        | Yersinia pesitis Detected<br>Unexpected Detected Results for <i>F. tularensis</i> <sup>l</sup> |
| Chikungunya virus (R80422 culture fluid)               |                        | As expected (only Chikungunya virus Detected)                                                  |
| Crimean-Congo Hemorrhagic fever virus                  |                        | As expected (only CCHF Detected)                                                               |
| Dengue                                                 | DENV-1 (Hawaii)        | As expected (only Dengue virus Detected)                                                       |
|                                                        | DENV-2 ( New Guinea C) | As expected (only Dengue virus Detected)                                                       |
|                                                        | DENV-3 (H87)           | As expected (only Dengue virus Detected)                                                       |
|                                                        | DENV-4 (H241)          | As expected (only Dengue virus Detected)                                                       |
| <i>Zaire ebolavirus</i>                                |                        | As expected (only Ebolavirus spp. Detected)                                                    |
| <i>Sudan ebolavirus</i>                                |                        | Ebolavirus spp. Detected<br>Unexpected Detected Result for <i>F. tularensis</i> <sup>l</sup>   |
| <i>Bundibugyo ebolavirus</i>                           |                        | As expected (only Ebolavirus spp. Detected)                                                    |
| <i>Tai Forest ebolavirus</i>                           |                        | As expected (only Ebolavirus spp. Detected)                                                    |
| <i>Reston ebolavirus</i>                               |                        | As expected (only Ebolavirus spp. Detected)                                                    |
| Lassa virus                                            |                        | As expected (only Lassa virus Detected)                                                        |
| Marburg Marburgvirus variant Musoke                    |                        | As expected (only Marburgvirus Detected)                                                       |
| Marburg marburgvirus (RAVN)                            |                        | As expected (only Marburgvirus Detected)                                                       |
| West Nile virus lineage 1                              |                        | As expected (only West Nile Virus Detected)                                                    |
| West Nile virus lineage 2                              |                        | As expected (only West Nile Virus Detected)                                                    |
| Yellow Fever virus                                     |                        | As expected (only Yellow Fever Virus Detected)                                                 |

| Pathogen                   |                                             | Results                                                                                                                              |
|----------------------------|---------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------|
| <i>Leishmania donovani</i> |                                             | As expected (only <i>Leishmania</i> spp. Detected)                                                                                   |
| <i>Plasmodium</i>          | <i>falciparum</i><br>(Pursat Cambodia 2011) | As expected ( <i>Plasmodium</i> spp. Detected and <i>P. falciparum</i> Detected)                                                     |
|                            | <i>knowlesi</i><br>(Strain H)               | <i>Plasmodium</i> spp Detected as expected.<br><br><i>Plasmodium vivax/ovale</i> detected at concentrations above 2.2E+03 copies/mL. |
|                            | <i>malariae</i><br>(DLS17-026015)           | As expected (only <i>Plasmodium</i> spp. Detected)                                                                                   |
|                            | <i>vivax</i><br>(11 Chesson)                | As expected ( <i>Plasmodium</i> spp. Detected and <i>P. vivax/ovale</i> Detected)                                                    |
|                            | <i>ovale</i><br>(Wallikeri)                 | As expected ( <i>Plasmodium</i> spp. Detected and <i>P. vivax/ovale</i> Detected)                                                    |

<sup>1</sup>Due to potential contamination event

Although no cross-reactivity with *P. malariae* was observed in this study, in silico analysis shows 1-3 mismatches between *P. malariae* and the *P. vivax/ovale* assay primers. Wet testing with *P. brasilianum* (a monkey *Plasmodium* thought to have descended from *P. malariae* and which has identical sequence as *P. malariae*) showed reactivity for the *P. vivax/ovale* assay at 1.6E+06 copies/mL.

Off-panel organisms were selected for testing based on a combination of several factors including:

- Relatedness to the species detected by the Global Fever Panel (near-neighbors)
- Clinical relevance (causing symptoms similar to the panel pathogens)
- likelihood of being present in blood as a co-infection based on a geographical region or specific population to which a panel pathogen is endemic
- genetic similarity to Global Fever Panel assay primers as determined by *in silico* analysis

BSL3/4 organisms and viruses that could not be obtained as inactivated stocks or purified nucleic acids were tested live in an appropriate facility (USAMRIID and The Center) to assess potential cross-reactivity.

Table 11 below lists the off-panel organisms that were tested at high concentrations (> 10<sup>6</sup> copies/mL) for potential cross-reactivity with the FilmArray Global Fever Panel.

**Table 11. Off-Panel Organisms Tested by the BioFire Global Fever Special Pathogens Panel**

| Bacteria                       |                               | Viruses      |                                  |
|--------------------------------|-------------------------------|--------------|----------------------------------|
| <i>Acinetobacter baumannii</i> | <i>Leptospira wolbachii</i>   | Adenovirus 1 | Omsk hemorrhagic fever virus     |
| <i>Bacillus brevis</i>         | <i>Leptospira yanagawae</i>   | Adenovirus 3 | O'nyong-nyong virus <sup>2</sup> |
| <i>Bacillus cereus</i>         | <i>Listeria monocytogenes</i> | Adenovirus 5 | Parvo virus                      |

|                                  |                                                                       |                                                           |                                                 |
|----------------------------------|-----------------------------------------------------------------------|-----------------------------------------------------------|-------------------------------------------------|
| <i>Bacillus circulans</i>        | <i>Mycobacterium tuberculosis</i> (avirulent)                         | Aura virus                                                | Powassan virus                                  |
| <i>Bacillus coagulans</i> Hammer | <i>Mycoplasma pneumoniae</i>                                          | Barmah Forest virus                                       | Rabies virus                                    |
| <i>Bacillus halodurans</i>       | <i>Neisseria meningitidis</i>                                         | Bunyamwera virus                                          | Rift Valley Fever Virus                         |
| <i>Bacillus licheniformis</i>    | <i>Proteus mirabilis</i>                                              | Coronavirus NL63                                          | Ross River virus                                |
| <i>Bacillus megaterium</i>       | <i>Pseudomonas aeruginosa</i>                                         | Crimean-Congo Hemorrhagic Fever Virus                     | Human respiratory syncytial virus               |
| <i>Bacillus mycoides</i>         | <i>Rickettsia typhi</i>                                               | Dugbe virus                                               | Rubella virus                                   |
| <i>Bacillus pumilus</i>          | <i>Salmonella enterica</i> subsp. <i>arizonae</i>                     | Eastern Equine Encephalitis Virus                         | Saint Louis encephalitis virus                  |
| <i>Bacillus subtilis</i>         | <i>Salmonella enterica</i> subsp. <i>bongori</i>                      | Ebolavirus (Zaire, Sudan, Bundibugyo, Tai Forest, Reston) | Sindbis virus                                   |
| <i>Bacillus thuringiensis</i>    | <i>Salmonella enterica</i> subsp. <i>diarizoniae</i>                  | Enterovirus, HEV-71                                       | Spondweni virus                                 |
| <i>Bacteroides fragilis</i>      | <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Dublin      | Epstein Barr virus                                        | Tickborne encephalitis virus                    |
| <i>Bordetella bronchiseptica</i> | <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Enteritidis | Flexal virus                                              | Una virus                                       |
| <i>Borrelia burgdorferi</i>      | <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Javiana     | Guanarito virus                                           | Usutu virus                                     |
| <i>Brucella melitensis</i>       | <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Manchester  | Hantaan virus                                             | Vaccinia virus                                  |
| <i>Burkholderia cepacia</i>      | <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Montevideo  | Hazara virus                                              | Varicella zoster virus                          |
| <i>Burkholderia mallei</i>       | <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Muenchen    | Hendra virus                                              | Venezuelan Equine Encephalomyelitis (VEE) virus |
| <i>Burkholderia pseudomallei</i> | <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Newport     | Hepatitis A Virus (HAV)                                   | Western Equine Encephalitis (WEE) virus         |
| <i>Chlamydomonas pneumoniae</i>  | <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Paratyphi   | Hepatitis B Virus (HBV)                                   | West Nile virus Lineage 1                       |
| <i>Clostridium bifermentans</i>  | <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Rubislaw    | Hepatitis C Virus (HCV)                                   | West Nile virus Lineage 2                       |
| <i>Clostridium perfringens</i>   | <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Saintpaul   | Herpes Simplex Virus 2 (HSV-2)                            | Yellow Fever virus                              |
| <i>Clostridium sordelli</i>      | <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Tennessee   | Human Parainfluenza Virus 1                               | Zika virus                                      |
| <i>Clostridium sporogenes</i>    | <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Thompson    | Human Parainfluenza Virus 3                               | <b>Fungi</b>                                    |
| <i>Clostridium perfringens</i>   | <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhi       | Hughes virus                                              | <i>Aspergillus fumigatus</i>                    |
| <i>Coxiella burnetii</i>         | <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium | Human herpesvirus 6B                                      | <i>Cryptococcus neoformans</i>                  |
| <i>Enterobacter aerogenes</i>    | <i>Salmonella enterica</i> subsp. <i>houteanae</i>                    | Human Immunodeficiency Virus 1                            | <b>Pathogenic Protozoa</b>                      |
| <i>Enterococcus faecalis</i>     | <i>Salmonella enterica</i> subsp. <i>indica</i>                       | Human Immunodeficiency Virus 2                            | <i>Babesia microti</i>                          |
| <i>Enterococcus faecium</i>      | <i>Salmonella enterica</i> subsp. <i>salamae</i>                      | Human T-lymphotropic virus type 1                         | <i>Crithidia fasciculata</i> <sup>3</sup>       |
| <i>Francisella hispaniensis</i>  | <i>Serratia marcescens</i>                                            | Human T-lymphotropic virus type 2                         | <i>Cyclospora cayetanensis</i>                  |

|                                                             |                                    |                                     |                                                  |
|-------------------------------------------------------------|------------------------------------|-------------------------------------|--------------------------------------------------|
| <i>Francisella persica</i>                                  | <i>Staphylococcus aureus</i>       | Influenza A H1N1-2009               | <i>Leishmania donovani</i>                       |
| <i>Francisella philomiragia</i>                             | <i>Streptococcus agalactiae</i>    | Influenza A H3N2                    | <b><i>Leptomonas seymouri</i><sup>4</sup></b>    |
| <i>Klebsiella oxytoca</i>                                   | <i>Streptococcus pneumoniae</i>    | Influenza B virus                   | <i>Schistosoma mansoni</i>                       |
| <i>Legionella pneumophila</i>                               | <i>Streptococcus pyogenes</i>      | Japanese Encephalitis Virus (JEV)   | <i>Toxoplasma gondii</i>                         |
| <b><i>L. amazonensis</i><sup>1</sup></b>                    | <i>Vibrio cholerae</i>             | Junin virus                         | <i>Trypanosoma brucei</i>                        |
| <b><i>L. braziliensis</i> Vianna<sup>1</sup></b>            | <i>Yersinia aldovae</i>            | Lassa virus                         | <i>Trypanosoma cruzi</i>                         |
| <b><i>L. major</i> IR173<sup>1</sup></b>                    | <i>Yersinia bercovieri</i>         | Machupo virus                       | <i>Trypanosoma rangeli</i>                       |
| <b><i>L. Mexicana</i><sup>1</sup></b>                       | <i>Yersinia enterocolitica</i>     | Marburg Marburgvirus variant Musoke | <b><i>Plasmodium berghei</i><sup>5</sup></b>     |
| <b><i>L. panamensis</i><sup>1</sup></b>                     | <i>Yersinia fredericksonii</i>     | Marburg Marburgvirus (RAVN)         | <b><i>Plasmodium brasilianum</i><sup>6</sup></b> |
| <b><i>L. tropica</i> (MHOM/AF/87/RUP)<sup>1</sup></b>       | <i>Yersinia intermedia</i>         | Mayaro virus                        | <b><i>Plasmodium cynomolgi</i><sup>7</sup></b>   |
| <b><i>L. tropica</i> (MHOM SU/58/strain-OD)<sup>1</sup></b> | <i>Yersinia kristensenii</i>       | Measles virus                       | <b><i>Plasmodium fieldi</i><sup>8</sup></b>      |
| <i>Leptospira biflexa</i>                                   | <i>Yersinia mollaretii</i>         | Metapneumovirus                     | <b><i>Plasmodium fragile</i><sup>9</sup></b>     |
| <i>Leptospira meyeri</i>                                    | <i>Yersinia pestis</i>             | Mopeia Virus                        | <b><i>Plasmodium inui</i><sup>10</sup></b>       |
| <i>Leptospira terpstrae</i>                                 | <i>Yersinia pseudotuberculosis</i> | Mumps Virus                         | <b><i>Plasmodium simiovale</i><sup>11</sup></b>  |
| <i>Leptospira vanthielii</i>                                | <i>Yersinia rohdei</i>             |                                     |                                                  |

<sup>1</sup>Assay reactivity is expected for these *Leishmania* species which may be present in the blood due to atypical visceral leishmaniasis.

<sup>2</sup>At high concentrations ( $\leq 1:1000$  dilution of stock), the o'nyong-nyong virus triggered a detected result for chikungunya virus. O'nyong-nyong and chikungunya virus infections can occur in the same geographic locations and present with similar symptoms. Appropriate warnings will be included in product literature.

<sup>3</sup>*Crithidia fasciculata* generated *Leishmania* spp. Detected result. It is a non-human infective trypanosomatid, however there have been very rare cases where a closely related *Crithidia* strain was found to be infective and act much like *Leishmania* spp.

<sup>4</sup>*Leptomonas seymouri* generated a *Leishmania* spp. Detected result. It is an opportunistic parasite in immunocompromised individuals particularly those infected with visceral leishmaniasis.

<sup>5</sup>*P. berghei* was reactive for both pan-species *Plasmodium* assay target and the *Plasmodium vivax/ovale* assay target at high concentrations ( $\leq 1:1000$  dilution of stock).

<sup>6</sup>*P. brasilianum* was reactive for both pan-species *Plasmodium* assay target and the *Plasmodium vivax/ovale* assay target at concentrations  $>1.6E+05$  copies/mL.

<sup>7</sup>*P. cynomolgi* produced reactive results for both the pan-species *Plasmodium* assay target and the *Plasmodium vivax/ovale* assay target. Cross-reactivity for both assay targets disappeared at concentrations  $\leq 2.6E-01$  copies/mL.

<sup>8</sup>*P. fieldi* exhibited cross-reactivity with both the *P. falciparum* spp. assay and the *P. vivax/ovale* target assay. Observed reactivity for both assays disappeared for analyte concentrations corresponding to  $\leq 5.8E+00$  copies/mL.

<sup>9</sup>*P. fragile* produced detected results for both the *Plasmodium* spp. assay target and the *P. vivax/ovale* assay targets. Reactivity with *P. vivax/ovale* assay disappeared at analyte concentrations corresponding to  $\leq 1.2E+03$  copies/mL.

<sup>10</sup>*P. inui* produced Detected results for both the pan-species *Plasmodium* assay target and the *P. vivax/ovale* assay target. Cross-reactivity with *P. vivax/ovale* disappeared at analyte concentrations corresponding to  $\leq 4.6E+04$  copies/mL.

<sup>11</sup>*P. simiovale* produced a Detected result for both the pan-species *Plasmodium* assay target and the *P. vivax/ovale* assay target. Cross-reactivity was observed for concentrations corresponding to  $\geq 4.2E+00$  copies/mL.

There were several instances of cross-reactivity observed during testing:

- 1) Assay reactivity is expected for other *Leishmania* species beyond *L. infantum* and *L. donovani*. In rare cases of atypical visceral leishmaniasis, these organisms may be detected in the blood of infected individuals.
- 2) Both *Leptomonas seymouri* and *Crithidia fasciculata* exhibited unexpected *Leishmania* spp. detected results.
- 3) At high concentrations, the o'nyong-nyong virus produces a chikungunya virus detected result. No cross-reactivity was observed when o'nyong-nyong virus was evaluated at concentrations below 1:1000 dilution of stock.

- 4) The majority of *Plasmodium* species evaluated as part of the off-panel cross-reactivity study are associated with non-human primate or rodent infections. *Plasmodium berghei*, *P. brasilianum*, *P. cynomolgi*, *P. fieldi*, *P. fragile*, *P. inui*, and *P. simiovale* are closely related to both *P. vivax* and *P. ovale* and were detected by both the Global Fever *Plasmodium* spp. and *P. vivax/ovale* assays at concentrations ranging from low inclusivity levels, to very high levels with greatly reduced sensitivity. These non-human isolates are unlikely to represent a significant risk of false positive results.

When *P. brasilianum* was evaluated for exclusivity, it was shown to cross-react with the *P. vivax* assay at  $1.6E+06$ , but not  $1.6E+05$  copies/mL. This non-human primate species of *Plasmodium* is thought to have evolved from *P. malariae* as recently as 500 years ago, and the two species share a high degree of sequence similarity. In silico analyses of the *P. vivax/ovale* assay indicate that *P. brasilianum* and *P. malariae* have identical mismatches to the *P. vivax/ovale* primers. For this reason, it is possible that *P. malariae* would also cross-react with the *Plas vivax* assay at  $\geq 1.6E+06$  copies/mL.

No other cross-reactivity was observed in wet testing.

In addition to the experimental testing, *in silico* analyses directed toward the potential to cross-react with specific pathogens that were unavailable for testing was also performed. This included Avalon virus, Bas-Congo virus, *Bacillus luciferensis*, *Chlamydia psittaci*, *Francisella mediasiatica*, Lymphocytic choriomeningitis virus, Middleburg virus, Murray Valley encephalitis virus, *Orientia chuto* (*tsutsugamushi*), Pirital virus, *Rickettsia prowazekii*, *Rickettsia rickettsii*, Sabia virus, Semliki Forest virus, Tonate virus, and Variola major. No expected cross-reactivity with off-panel pathogens were predicted by *in silico* analysis.

### *Interfering Substances*

Potentially interfering substances that could be present in whole blood or introduced during specimen collection and testing were evaluated for their effect on the BioFire Global Fever Special Pathogens Panel performance. Each substance was added to contrived samples containing representative FilmArray Global Fever Special Pathogens Panel organisms (*Leptospira interrogans*, Dengue virus Type 3, *P. falciparum* and *Leishmania donovani*) at concentrations equivalent to approximately  $3 \times \text{LoD}$ . The concentrations of endogenous and exogenous substances tested were based on a reference concentration of “normal” to “high” levels expected to be present in clinical specimens.

The following two tables show the results for tested endogenous and exogenous substances, and for technique specific substances and anticoagulants, respectively.

**Table 12. Results for Potentially Interfering Substances Tested on the BioFire Global Fever Special Pathogens Panel (Endogenous and Exogenous Substances)**

| Endogenous Substances    | Concentration Tested <sup>a</sup> | Results                  |
|--------------------------|-----------------------------------|--------------------------|
| Albumin                  | 60.0 mg/mL                        | No interference observed |
| Bilirubin (Conjugated)   | 0.41 mg/mL                        | No interference observed |
| Bilirubin (Unconjugated) | 0.41 mg/mL                        | No interference observed |

| Cholesterol (total)                         | 4.2 mg/mL                         | No interference observed |
|---------------------------------------------|-----------------------------------|--------------------------|
| Glucose                                     | 10.1 mg/mL                        | No interference observed |
| Hemoglobin                                  | 137.0 mg/mL                       | No interference observed |
| Immunoglobulins                             | 60 mg/mL                          | No interference observed |
| Triglycerides                               | 15.1 mg/mL                        | No interference observed |
| Human cells<br>(K-562 Human Leukemia Cells) | 6.1E+06 cells/mL                  | No interference observed |
| Exogenous Substances                        | Concentration Tested <sup>a</sup> | Results                  |
| Artemether-Lumefantrine                     | 0.0004 mg/mL                      | No interference observed |
| Atovaquone                                  | 0.005 mg/mL                       | No interference observed |
| Proguanil                                   | 0.001 mg/mL                       | No interference observed |
| Mefloquine                                  | 0.0017 mg/mL                      | No interference observed |
| Amphotericin B                              | 0.002 mg/mL                       | No interference observed |
| Pentamidine                                 | 0.0015 mg/mL                      | No interference observed |
| Fluconazole                                 | 0.026 mg/mL                       | No interference observed |
| Amoxicillin                                 | 0.062 mg/mL                       | No interference observed |
| Azithromycin                                | 0.011 mg/mL                       | No interference observed |
| Ceftriazone                                 | 1.0 mg/mL                         | No interference observed |
| Ciprofloxacin                               | 0.012 mg/mL                       | No interference observed |
| Clindamycin                                 | 0.055 mg/mL                       | No interference observed |
| Doxycycline                                 | 0.02 mg/mL                        | No interference observed |
| Gentamicin                                  | 0.036 mg/mL                       | No interference observed |
| Meropenem                                   | 0.39 mg/mL                        | No interference observed |
| Sulfamethoxazole                            | 0.38 mg/mL                        | No interference observed |
| Vancomycin                                  | 0.12 mg/mL                        | No interference observed |
| Cycloserine                                 | 75.0 mg/mL                        | No interference observed |
| Isoniazid                                   | 0.06 mg/mL                        | No interference observed |
| Oseltamivir                                 | 0.0005 mg/mL                      | No interference observed |
| Ribavirin                                   | 0.011 mg/mL                       | No interference observed |
| Tenofovir                                   | 0.001 mg/mL                       | No interference observed |
| Acetaminophen                               | 0.16 mg/mL                        | No interference observed |
| Aspirin                                     | 0.03 mg/mL                        | No interference observed |
| Ibuprofen                                   | 0.22 mg/mL                        | No interference observed |
| Prednisone                                  | 0.0001 mg/mL                      | No interference observed |
| Prednisolone                                | 1.2 mg/mL                         | No interference observed |
| Cortisone                                   | 0.001 mg/mL                       | No interference observed |
| Artesunate                                  | 0.1 mg/mL                         | No interference observed |

<sup>a</sup>Concentrations of interfering substances were based on guidelines contained in CLSI guidelines for interference testing (EP07 and EP37) when available.

**Table 13. Results for Potentially Interfering Substances Tested on the BioFire Global Fever Special Pathogens Panel (Disinfectants, Solvents, and Anticoagulants)**

| Technique Specific Substances | Concentration Tested      | Results                  |
|-------------------------------|---------------------------|--------------------------|
| Bleach                        | 1% (v/v) or 525 ppm NaOCl | No interference observed |
| Povidone-iodine               | 1% (v/v)                  | No interference observed |
| Ethanol                       | 2% (v/v)                  | No interference observed |
| TRIzol                        | 2-3% (v/v)                | Potentially Interfering  |

|                                       |                                                             |                          |
|---------------------------------------|-------------------------------------------------------------|--------------------------|
| DMSO <sup>1</sup>                     | 2% (v/v)                                                    | No interference observed |
| Methanol <sup>1</sup>                 | 2% (v/v)                                                    | No interference observed |
| Saline <sup>1</sup>                   | 2% (v/v)                                                    | No interference observed |
| Chloroform <sup>1</sup>               | 2% (v/v)                                                    | No interference observed |
| Acetone <sup>1</sup>                  | 2% (v/v)                                                    | No interference observed |
| Hydrochloric Acid <sup>1</sup>        | 0.0005 N                                                    | No interference observed |
| <b>Anticoagulant</b>                  | <b>Concentration Tested</b>                                 | <b>Results</b>           |
| Citrate (sodium)                      | ~0.32 % (10.9 mmol/L)                                       | No interference observed |
| K·EDTA in excess (5x) <sup>2</sup>    | ~9 mg/mL                                                    | No interference observed |
| Na·Heparin                            | ~19 USP/mL                                                  | Potentially Interfering  |
| Acid-citrate-dextrose (ACD)           | ~2.2 g/L citrate<br>0.8 g/L citric acid<br>2.5 g/L dextrose | No interference observed |
| Sodium polyanethenole sulfonate (SPS) | 0.72 mg/mL                                                  | No interference observed |
| Serum separation tubes                | N/A                                                         | No interference observed |

<sup>1</sup>Testing of these specific substances was performed to account for their use as solvents for resuspension of other potential interferants.

<sup>2</sup>Testing was performed to evaluate potential interference caused by a short blood draw and a collection tube that thus contained higher anticoagulant concentration

Dengue virus, *Leptospira* spp., and *Plasmodium* spp. vaccines were not evaluated in this study but are predicted to be reactive with the corresponding Global Fever Panel assay targets.

All evaluated exogenous substances exhibited no interference effect. Among technique specific substances, initial testing of TRIzol exhibited an interference effect wherein 2/3 *P. falciparum* replicates were incorrectly reported as a Not Detected result. Subsequent retesting at the same interferent level and additionally with samples containing 3% TRIzol (v/v) did not produce unexpected assay results but may result in delayed Cp values. Similarly, initial testing with Heparin resulted in two unexpected Not Detected results for a single analyte with only 3/5 replicates positive. However, repeat testing at the same heparin concentration successfully met acceptance criteria with 4/5 replicates positive. These data demonstrate that Heparin levels near the evaluated concentration may be inhibitory for analytes near the LoD.

#### 4. Assay Reportable Range:

Not applicable.

#### 5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

##### *Internal Controls:*

Two internal controls are included in each BioFire Global Fever Special Pathogens Panel pouch:

- RNA Process Control: The RNA Process Control assay targets an RNA transcript from the yeast *Schizosaccharomyces pombe*. The yeast is present in the pouch in a freeze-dried form and becomes rehydrated when sample is loaded. The control

material is carried through all stages of the test process, including lysis, nucleic acid purification, reverse transcription, 1st stage PCR, dilution, 2nd stage PCR, and DNA melting. A positive control result indicates that all steps carried out in the BioFire Global Fever Special Pathogens Panel pouch were successful.

- PCR2 Control: The PCR2 Control assay detects a DNA target that is dried into wells of the array along with the corresponding primers. A positive result indicates that 2nd stage PCR was successful.

Both control assays must be positive for the test run to pass. If either control fails, the Controls field of the test report will display “Failed” and all results will be listed as “Invalid”. If the controls fail, the sample should be retested using a new pouch.

#### Recommended External Controls:

External controls are not provided with the BioFire Global Fever Special Pathogens Panel but are recommended in the package insert. External controls should be used in accordance with laboratory protocols and the appropriate accrediting organization requirements, as applicable. Molecular grade water or saline can be used as an external negative control. Previously characterized positive samples or negative samples spiked with well-characterized organisms can be used as external positive controls.

BioFire Defense provides an external positive and negative assayed quality control kit to monitor the performance of *in vitro* laboratory nucleic acid testing procedures for the qualitative detection of the BioFire Global Fever Special Pathogens Panel performed on FilmArray systems. The positive external control is a surrogate control material comprised of dried synthetic DNA (positive only) in buffer and stabilizer, supplied in a FilmArray Injection Vial that is used directly with the BioFire Global Fever Special Pathogens Panel. The BioFire SHIELD Control Kit for the BioFire Global Fever Special Pathogens Panel is composed of two controls: BioFire Global Fever Special Pathogens Panel Positive External Control Material (Positive ECM) and BioFire Global Fever Special Pathogens Panel Negative External Control Material (Negative ECM). The DNA in the Positive ECM includes DNA segments to assess the presence of each individual assay in the BioFire Global Fever Special Pathogens Panel listed above. There is no DNA in the Negative ECM. The Global Fever Panel Control Kit contains no biological hazards and is 100% non-infectious. To use the product, the operator opens and uses the FilmArray Injection Vial in place of the Sample Injection Vial, and otherwise runs the test according to protocol. This control is shipped and stored at 18-28 °C.

The external control kit is available for purchase directly from BioFire Defense.

#### *Quantification of nucleic acid derived from live or inactivated viral and bacterial cultures*

Quantification using the genesig qPCR kits was performed at two different locations, 1) USAMRIID – live Chikungunya virus stocks, 2) BioFire Defense – live and inactivated bacterial, viral, and protozoan stocks.

Organisms evaluated at BioFire Defense were obtained from BEI Resources, ATCC, Leptospirosis Reference Centre AMC, Centers for Disease Control and Prevention, University of Texas Medical Branch World Reference Center for Emerging Viruses and Arboviruses, Discovery Life Sciences, and Zeptomatrix. Verification of the stocks, whenever available, was provided with a Certificate of Analysis (CofA) from the source. The majority of organisms obtained did not come quantified or came without any enumeration value.

Nucleic acids from the bacterial and viral stocks were extracted using Qiagen mini-kits (bacteria - QIAamp DNA Mini Kit cat#51306; viruses - Qiagen QIAamp Viral RNA Mini Kit cat#52906). All virus stocks were treated with Trizol reagent prior to extraction. The nucleic acid concentration of each extract was then determined using the commercially available genesig qPCR assay kits from Primerdesign Ltd, Southampton, UK. The Bio Rad CFX96 Touch Real-Time PCR Detection System and the Applied Biosystems Real-Time PCR Instrument were used to perform the qPCR assays at BioFire Diagnostics/BioFire Defense and USAMRIID, respectively. Cycling conditions were as recommended by Primerdesign Ltd and are shown below in Table 14. Fluorescence data were collected in the FAM channel.

**Table 14. Amplification Cycling Conditions for the Genesig qPCR Kits**

|           | Step                               | Time    | Temp. |
|-----------|------------------------------------|---------|-------|
|           | Reverse Transcription <sup>1</sup> | 10 min. | 42 °C |
|           | Enzyme activation                  | 2 min.  | 95 °C |
| 50 Cycles | Denaturation                       | 10 sec. | 95 °C |
|           | Data Collection                    | 60 sec. | 60 °C |

<sup>1</sup> Reverse Transcription step was only performed with RNA organisms.

Both DNA and RNA target nucleic acid from organism stocks was extracted using the MagNA Pure Compact Instrument. The nucleic acid concentration of the extract (Unknown) was then determined using commercially available quantitative real-time PCR assay Standard Kits from genesig, Primerdesign Ltd, Southampton, UK.

**Table 15. Quantified Organisms and the Genesig qPCR Assay Kits Targets and the BioFire Global Fever Special Pathogens Panel Targets**

| FilmArray Global Fever Panel Analyte | Quantified Species | Strain                 | Genesig qPCR Kit Gene Target | FilmArray Global Fever Panel Target(s) |
|--------------------------------------|--------------------|------------------------|------------------------------|----------------------------------------|
| <b>Bacteria</b>                      |                    |                        |                              |                                        |
| <i>Bacillus anthracis</i>            |                    | Ames35                 | hypothetical protein         | BA5345                                 |
|                                      |                    | Sterne 34F2            |                              |                                        |
|                                      |                    | UM23                   |                              |                                        |
|                                      |                    | Weybridge              |                              |                                        |
| <i>Francisella tularensis</i>        |                    | Holarctica, LVSR       | sdhA                         | sodC<br>vgrG                           |
|                                      |                    | Holarctica, Type B LVS |                              |                                        |
|                                      |                    | Novicida, KM14S        |                              |                                        |
|                                      |                    | Novicida, CG62         |                              |                                        |
|                                      |                    | Scherm                 |                              |                                        |
|                                      |                    | WY96                   |                              |                                        |
|                                      |                    | Holarctica, LVS        |                              |                                        |
|                                      |                    | Holarctica             |                              |                                        |

| FilmArray Global<br>Fever Panel Analyte  | Quantified<br>Species | Strain                         | Genesig qPCR<br>Kit Gene<br>Target | FilmArray Global Fever Panel Target(s)                             |
|------------------------------------------|-----------------------|--------------------------------|------------------------------------|--------------------------------------------------------------------|
|                                          |                       | Holarctica, 425                |                                    |                                                                    |
|                                          |                       | Holarctica, HD                 |                                    |                                                                    |
|                                          |                       | Holarctica, VT68               |                                    |                                                                    |
|                                          |                       | Novicida F6168                 |                                    |                                                                    |
|                                          |                       | U112, GA993550                 |                                    |                                                                    |
| <i>Leptospira</i> spp.                   | <i>kirschneri</i>     | 200701401                      | Lipl32                             | groES                                                              |
|                                          |                       | 3522 C (Cynopteri)             |                                    |                                                                    |
|                                          | <i>interrogans</i>    | Serovar<br>icterohaemorrhagiae |                                    |                                                                    |
|                                          |                       | HAI0156 (Copenhagen)           |                                    |                                                                    |
|                                          |                       | L495 (Manilae)                 |                                    |                                                                    |
|                                          | <i>alexanderi</i>     | L 60 (Manhao 3)                |                                    |                                                                    |
|                                          | <i>santarosai</i>     | LT 821 (Shermani)              |                                    |                                                                    |
|                                          | <i>kmetyi</i>         | Bejo-Iso9T (Malaysia)          |                                    |                                                                    |
|                                          | <i>noguchii</i>       | CZ 214T (Panama)               |                                    |                                                                    |
|                                          | <i>borgpetersenii</i> | Veldrat Bataviae 46            |                                    |                                                                    |
|                                          | <i>weilii</i>         | Celledoni 20160426             |                                    |                                                                    |
|                                          |                       | A 102 (Mengrun)                |                                    |                                                                    |
|                                          |                       | 6712 (Hainan)                  |                                    |                                                                    |
|                                          |                       | H 27 (Hekou)                   |                                    |                                                                    |
|                                          |                       | LT 89-68 (Vughia)              |                                    |                                                                    |
|                                          | 94-79970/3 (Topaz)    |                                |                                    |                                                                    |
| <i>meyeri</i>                            | ICF                   |                                |                                    |                                                                    |
| <i>Yersinia pestis</i>                   | Orientalis            | A1122                          | pla                                | put. chaperone protein (caf1M)<br>pst                              |
|                                          |                       | Shasta                         |                                    |                                                                    |
|                                          |                       | Java 9                         |                                    |                                                                    |
|                                          |                       | PBM19                          |                                    |                                                                    |
|                                          |                       | PY-013                         |                                    |                                                                    |
|                                          | Antigua               | PH 80/63                       |                                    |                                                                    |
|                                          |                       | Antigua                        |                                    |                                                                    |
|                                          |                       | Nairobi                        |                                    |                                                                    |
|                                          |                       | Angola                         |                                    |                                                                    |
|                                          | Medievalis            | Harbin 35                      |                                    |                                                                    |
|                                          |                       | KIM5                           |                                    |                                                                    |
|                                          |                       | Nicholisk 41                   |                                    |                                                                    |
| Pestoides B                              |                       |                                |                                    |                                                                    |
| Viruses                                  |                       |                                |                                    |                                                                    |
| Chikungunya Virus                        |                       | R80422                         | Non-structural<br>Protein 2        | Nonstructural polyprotein of CHIKVgp1<br>3' UTR region of CHIKVgp1 |
|                                          |                       | St. Martin 2013                |                                    |                                                                    |
|                                          |                       | DHS4263                        |                                    |                                                                    |
|                                          |                       | B8653                          |                                    |                                                                    |
|                                          |                       | Indo 23574                     |                                    |                                                                    |
| Crimean-Congo Hemorrhagic Fever<br>Virus |                       | IbAr10200                      | Nucleocapsid<br>region gene        | L-Segment                                                          |
| Dengue Virus                             | Serotype 1            | Hawaii                         | 3'UTR                              | Envelope Protein                                                   |
|                                          |                       | 276RK1                         |                                    |                                                                    |
|                                          |                       | Strain 12150                   |                                    |                                                                    |
|                                          |                       | BC89/94                        |                                    |                                                                    |
|                                          |                       | 228690                         |                                    |                                                                    |
|                                          |                       | VN/BID-V1792/2007              |                                    |                                                                    |
|                                          |                       | SL-6-6-04                      |                                    |                                                                    |
|                                          | Serotype 2            | New Guinea C                   |                                    |                                                                    |
|                                          |                       | VN/BID-V1002/2006              |                                    |                                                                    |
|                                          |                       | DakArA1247                     |                                    |                                                                    |
|                                          |                       | BC102/94                       |                                    |                                                                    |
|                                          |                       | 429557                         |                                    |                                                                    |
|                                          |                       | 1349                           |                                    |                                                                    |
|                                          |                       | ArA6894                        |                                    |                                                                    |
|                                          |                       | DKA 811                        |                                    |                                                                    |

| FilmArray Global<br>Fever Panel Analyte | Quantified<br>Species | Strain                        | Genesig qPCR<br>Kit Gene<br>Target | FilmArray Global Fever Panel Target(s)                                               |
|-----------------------------------------|-----------------------|-------------------------------|------------------------------------|--------------------------------------------------------------------------------------|
|                                         | Serotype 3            | H87                           |                                    |                                                                                      |
|                                         |                       | VN/BID-V1329/2006             |                                    |                                                                                      |
|                                         |                       | BC188/97                      |                                    |                                                                                      |
|                                         |                       | 271242                        |                                    |                                                                                      |
|                                         |                       | C0360/94                      |                                    |                                                                                      |
|                                         | Serotype 4            | H241                          |                                    |                                                                                      |
|                                         |                       | 703                           |                                    |                                                                                      |
|                                         |                       | D85-019                       |                                    |                                                                                      |
|                                         |                       | BC13/97                       |                                    |                                                                                      |
|                                         |                       | BC287/97                      |                                    |                                                                                      |
|                                         |                       | BC258/97                      |                                    |                                                                                      |
|                                         |                       | PR 06-65-740                  |                                    |                                                                                      |
| Ebola virus                             | Sudan                 | Boniface                      | Nucleoprotein                      | RDRP<br>NP<br>Polymerase cofactor (VP35)                                             |
|                                         | Zaire                 | Gueckedou                     |                                    |                                                                                      |
|                                         |                       | Kikwit ‘95                    |                                    |                                                                                      |
|                                         |                       | Gabon                         |                                    |                                                                                      |
|                                         |                       | Luebo                         |                                    |                                                                                      |
| Lassa virus                             |                       | Josiah                        | GP                                 | S-segment                                                                            |
| Marburgvirus                            |                       | Musoke                        | VP35                               | Nucleoprotein                                                                        |
|                                         |                       | Voegel                        |                                    |                                                                                      |
|                                         |                       | Kenya                         |                                    |                                                                                      |
|                                         |                       | Ci67                          |                                    |                                                                                      |
| Ravn virus                              |                       | Ravn                          |                                    |                                                                                      |
| West Nile Virus                         |                       | B-956 Uganda (lin. 2)         | 5’ UTR                             | NS5                                                                                  |
|                                         |                       | B-956 (lin. 2)                |                                    |                                                                                      |
|                                         |                       | Bird 114 (lin. 1)             |                                    |                                                                                      |
|                                         |                       | NY 2001-6263 (lin. 1)         |                                    |                                                                                      |
|                                         |                       | 1986 (lin. 1)                 |                                    |                                                                                      |
|                                         |                       | Bz NY99 (lin. 1)              |                                    |                                                                                      |
|                                         |                       | Eg101 (lin. 1)                |                                    |                                                                                      |
| Yellow Fever Virus                      |                       | 17D                           | polyprotein                        | Capsid protein C                                                                     |
|                                         |                       | Asibi                         |                                    |                                                                                      |
| Protozoa                                |                       |                               |                                    |                                                                                      |
| Leishmania spp.                         | L. donovani           | 1S                            | cytb                               | 18S                                                                                  |
|                                         |                       | 9515                          |                                    |                                                                                      |
|                                         | L. infantum           | mhom/tn/80/ipt-1              |                                    |                                                                                      |
|                                         | L. major              | IR173                         |                                    |                                                                                      |
|                                         | L. mexicana           | MHOM/BZ/74/PP75               |                                    |                                                                                      |
|                                         | L. chagasi            | MHOM/BR/74/PP75               |                                    |                                                                                      |
|                                         | L. venezuelensis      | MHOM-VE/80/H-16               |                                    |                                                                                      |
|                                         | L. tropica            | NIH RUP                       |                                    |                                                                                      |
| MHOM/SU/58/strain-OD                    |                       |                               |                                    |                                                                                      |
| Plasmodium spp.                         | P.falciparum          | IPC 4884 Pursat Cambodia 2011 | 18S ribosome                       | Genus: Upstream to cytochrome oxidase III<br>Species: Pfmdr-1                        |
|                                         |                       | SenTh021.09                   |                                    |                                                                                      |
|                                         |                       | St. Lucia                     |                                    |                                                                                      |
|                                         |                       | Tanaznia, 02000708            |                                    |                                                                                      |
|                                         | P. vivax              | Chesson                       |                                    | Genus: Upstream to cytochrome oxidase III<br>Species: Cytochrome b                   |
|                                         |                       | Panama                        |                                    |                                                                                      |
|                                         | P. ovale              | Wallikeri                     |                                    | Genus: Upstream to cytochrome oxidase III<br>Species: Reticulocyte binding protein 2 |
|                                         | P. knowlesi           | Strain H                      |                                    | Genus: Upstream to cytochrome oxidase III                                            |
|                                         | P. malariae           | Unknown                       |                                    | Genus: Upstream to cytochrome oxidase III                                            |

## Specimen Transport and Storage (Specimen Stability)

Stability for whole blood specimens was evaluated to support labeling recommendations for storage of samples at room temperature for up to 24 hours, or at 2-8°C for up to seven days, or in an ultra-low temperature freezer ( $\leq -70^{\circ}\text{C}$ ). These storage conditions are consistent with or exceed standard storage and transport conditions and times for most laboratory testing of clinical human whole blood specimens. Testing was conducted using samples composed of human WB contrived with representative GF Panel analytes in two mixes at a concentration of  $3\times$  their limit of detection (LoD). Samples were tested immediately (ten replicates) after sample preparation as a no storage control or stored at the appropriate condition for various amounts of time (one day, three days, or seven days) prior to testing of additional 10 replicates per sample per storage condition.

As shown in Table 16 below, study results demonstrated 10/10 replicates positive for each evaluated storage condition for all analytes. All *P. falciparum* contrived specimens were reactive by both the *Plasmodium* spp. and *Plasmodium falciparum* assays. Mean Cp values for all analytes were also consistent between the control and stored samples thereby further supporting the storage claims.

**Table 16. Summary of Organism Detection in Whole Blood for All Storage Conditions Tested**

| Organism/Analyte<br>(Strain)      | Concentrati<br>on Tested          | # Detected / # Tested      |                     |                  |                      |       |       |                          |
|-----------------------------------|-----------------------------------|----------------------------|---------------------|------------------|----------------------|-------|-------|--------------------------|
|                                   |                                   | No<br>Storage<br>(Control) | Ambient Storage     |                  | Refrigerated Storage |       |       | Ultra-<br>low<br>freezer |
|                                   |                                   |                            | 18-21°C<br>for 24 h | 30°C for<br>24 h | Day 1                | Day 3 | Day 7 |                          |
| BACTERIA                          |                                   |                            |                     |                  |                      |       |       |                          |
| <i>Leptospira<br/>interrogans</i> | 1.0E+03<br>copies/mL<br>3 x LoD   | 10/10                      | 10/10               | 10/10            | 10/10                | 10/10 | 10/10 | 10/10                    |
| VIRUSES                           |                                   |                            |                     |                  |                      |       |       |                          |
| Dengue virus                      | 1.0E+03<br>copies/mL<br>3 x LoD   | 10/10                      | 10/10               | 10/10            | 10/10                | 10/10 | 10/10 | 10/10                    |
| PROTOZOA                          |                                   |                            |                     |                  |                      |       |       |                          |
| <i>Leishmania<br/>donovani</i>    | 3.0E+01<br>copies/mL<br>3 x LoD   | 10/10                      | 10/10               | 10/10            | 10/10                | 10/10 | 10/10 | 10/10                    |
| <i>Plasmodium<br/>falciparum</i>  | 2.7E+02<br>copies/mL<br>1.5 x LoD | 10/10                      | 10/10               | 10/10            | 10/10                | 10/10 | 10/10 | 10/10                    |

## Fresh vs. Frozen Study

Frozen specimens were evaluated as part of the clinical validation of the Global Fever Special Pathogens Panel due to circumstances that did not permit immediate testing. To determine whether specimen freezing adversely affects analyte detection by the Global Fever Panel, a total of 60 contrived specimens and 43 previously tested prospective clinical specimens were evaluated both fresh and after freezing at  $\leq -70^{\circ}\text{C}$  for at least five days. Contrived specimens were prepared with multiple analytes spiked at concentrations near 3 x

and 5 x LoD in clinical matrix. Contrived specimens were retested after having been frozen for 6-29 days. Clinical specimens were retested after having been frozen for 111-406 days. Overall agreement of the BioFire Global Fever Special Pathogens Panel results between fresh and frozen specimens were evaluated as PPA and NPA and results are shown in the tables below.

**Table 17. Performance Comparison of Fresh vs Frozen Contrived Specimens**

| Organism/Analyte<br>(Strain) | PPA              |      |           | NPA               |      |           |
|------------------------------|------------------|------|-----------|-------------------|------|-----------|
|                              | TP / (TP+<br>FN) | %    | 95% CI    | TN / (TN<br>+ FP) | %    | 95% CI    |
| <b>BACTERIA</b>              |                  |      |           |                   |      |           |
| <i>Bacillus anthracis</i>    | 16/16            | 100% | 81.6-100% | 44/44             | 100% | 92.0-100% |
| <i>Leptospira</i> spp.       | 16/16            | 100% | 81.6-100% | 44/44             | 100% | 92.0-100% |
| <b>VIRUSES</b>               |                  |      |           |                   |      |           |
| Dengue virus                 | 17/17            | 100% | 81.6-100% | 43/43             | 100% | 91.8-100% |
| <b>PROTOZOA</b>              |                  |      |           |                   |      |           |
| <i>Leishmania</i> spp.       | 17/17            | 100% | 81.6-100% | 43/43             | 100% | 91.8-100% |
| <i>Plasmodium falciparum</i> | 4/4              | 100% | 51.0-100% | 56/56             | 100% | 93.6-100% |
| <i>P. vivax/ovale</i>        | 7/7              | 100% | 64.6-100% | 53/53             | 100% | 93.2-100% |
| <i>Plasmodium</i> spp.       | 17/17            | 100% | 64.6-100% | 43/43             | 100% | 91.8-100% |

**Table 18. Performance Comparison of Fresh vs. Frozen Clinical Specimens**

| Organism/Analyte<br>(Strain)        | PPA                |      |           | NPA               |       |            |
|-------------------------------------|--------------------|------|-----------|-------------------|-------|------------|
|                                     | TP / (TP+<br>FN)   | %    | 95% CI    | TN / (TN<br>+ FP) | %     | 95% CI     |
| <b>BACTERIA</b>                     |                    |      |           |                   |       |            |
| <i>Leptospira</i> spp.              | 5/5                | 100% | 56.6-100% | 38/38             | 100%  | 90.8-100%  |
| <b>VIRUSES</b>                      |                    |      |           |                   |       |            |
| Dengue virus (Serotypes 1, 2, 3, 4) | 17/17              | 100% | 81.6-100% | 26/26             | 100%  | 87.1-100%  |
| Chikungunya Virus                   | 5/5                | 100% | 56.6-100% | 38/38             | 100%  | 90.8-100%  |
| <b>PROTOZOA</b>                     |                    |      |           |                   |       |            |
| <i>Plasmodium falciparum</i>        | 9/9                | 100% | 70.1-100% | 33/34             | 97.1% | 85.1-99.5% |
| <i>P. vivax/ovale</i>               | 10/10              | 100% | 72.2-100% | 32/33             | 97.0% | 84.7-99.5% |
| <i>Plasmodium</i> spp.              | 16/16 <sup>a</sup> | 100% | 80.6-100% | 27/27             | 100%  | 87.5-100%  |

<sup>a</sup>Three of the 16 *Plasmodium* spp.-positive fresh specimens were positive for both *P. falciparum* and *P. vivax/ovale*

<sup>b</sup>One specimen negative for *P. falciparum* assay when tested fresh was positive for *P. falciparum* when tested after freezing.

<sup>c</sup>One specimen that was negative for the *P. vivax/ovale* assay when tested fresh was positive for *P. vivax/ovale* when tested after freezing

## 6. Detection Limit:

A study to establish the detection limit of the BioFire Global Fever Special Pathogens Panel was conducted utilizing whole blood samples contrived with isolates of viruses, bacteria, and protozoa detected by the test. Specifically, testing utilized a combination of live and inactivated organisms prepared in multi-spiked mixes that contain 4-5 individual target organisms as described below:

**Table 19. Composition of Organism Mixes used in GF Special Pathogens Panel LoD Testing**

| Mix | Organism                              | Mix | Organism                                    |
|-----|---------------------------------------|-----|---------------------------------------------|
| A   | Chikungunya virus                     | E   | Dengue virus (DENV-2_1)                     |
|     | Dengue virus (DENV3)                  |     | Bundibugyo <i>ebolavirus</i>                |
|     | <i>Plasmodium falciparum</i>          |     | <i>Yersinia Pestis</i>                      |
| B   | Sudan <i>ebolavirus</i>               | F   | West Nile virus lineage 1                   |
|     | Dengue virus (DENV-1)                 |     | <i>Marburg marburgvirus</i> (Ravn virus)    |
|     | <i>Plasmodium vivax</i>               |     | Yellow fever virus                          |
|     | <i>Francisella tularensis</i>         |     | Lassa virus                                 |
| C   | Crimean-Congo hemorrhagic fever virus | G   | Reston <i>ebolavirus</i>                    |
|     | Dengue virus (DENV-4)                 |     | <i>Plasmodium ovale</i>                     |
|     | Zaire <i>ebolavirus</i>               |     | <i>Bacillus anthracis</i>                   |
|     | <i>Leishmania donovani</i>            |     | <i>Marburg Marburgvirus</i> (Marburg virus) |
|     | <i>Plasmodium malariae</i>            |     | <i>Plasmodium knowlesi</i>                  |
| D   | <i>Leptospira interrogans</i>         | H   | <i>Tai Forest ebolavirus</i>                |
|     | Dengue virus (DENV-2_2)               |     | <i>Marburg Marburgvirus</i> (Ravn virus)    |
|     | Tai Forest <i>ebolavirus</i>          |     | West Nile virus lineage 1                   |
|     | West Nile virus lineage 2             |     |                                             |

The LoD was first estimated by testing dilutions of contrived whole blood samples containing known dilutions of organisms bracketing an initial LoD concentration based on early development testing. Confirmation of LoD was achieved by testing 20 replicates of a contrived sample containing analyte at the estimated LoD. LoD was successfully confirmed when the organism was detected in at least 19 of the 20 of the replicates tested over three days using three pouch lots. Complete results for individual analytes included on the BioFire Global Fever Special Pathogens Panel are included in Table 7 below.

**Table 20. Limit of Detection for BioFire Global Fever Special Pathogens Panel Analytes**

| Global Fever Panel Test Result        | Species/Strain Tested                      | LoD                       |                                                  |
|---------------------------------------|--------------------------------------------|---------------------------|--------------------------------------------------|
|                                       |                                            | Based on qPCR (copies/mL) | Based on Provided Stock Concentration (units/mL) |
| BACTERIA                              |                                            |                           |                                                  |
| <i>Bacillus anthracis</i>             | Ames35 (Live)                              | 4.2E+01                   | N/A <sup>1</sup>                                 |
|                                       | Ames (Live)                                | 6.4E+01 <sup>2</sup>      | 3.5E+00 CFU/mL                                   |
| <i>Francisella tularensis</i>         | SCHU S4 (Inactivated)                      | 1.2E+03                   | N/A <sup>1</sup>                                 |
|                                       | subsp. tularensis Schu (Live)              | 1.2E+01 <sup>2</sup>      | 2.1E+02 CFU/mL                                   |
| <i>Leptospira interrogans</i>         | Icterohaemorrhagiae Budapest               | 3.4E+02                   | N/A <sup>1</sup>                                 |
| <i>Yersinia pestis</i>                | A1122 (Live)                               | 1.3E+02                   | N/A <sup>1</sup>                                 |
|                                       | CO92 (Live)                                | 1.5E+02 <sup>2</sup>      | 3.0E+01 CFU/mL                                   |
| VIRUSES                               |                                            |                           |                                                  |
| Chikungunya virus                     | R80422 (heat inactivated)                  | 5.5E+02                   | 3.6E-01                                          |
|                                       | B8635 (live)                               | 5.5E+02 <sup>2</sup>      | 4.4E+01 PFU/mL                                   |
|                                       | Indo23574 (live)                           | 5.5E+02 <sup>2</sup>      | 9.0E+01 PFU/mL                                   |
| Crimean-Congo hemorrhagic fever virus | Strain IbAr10200 (Inactivated)             | 6.4E+00                   | N/A <sup>1</sup>                                 |
|                                       | Strain IbAr10200 (Live)                    | 6.4E+02 <sup>2</sup>      | 2.9E+03 PFU/mL                                   |
| Dengue Virus 1                        | Hawaii                                     | 2.2E+02                   | N/A <sup>1</sup>                                 |
| Dengue Virus 2                        | New Guinea C                               | 3.4E+02                   | N/A <sup>1</sup>                                 |
|                                       | DakArA1247                                 | 2.7E+03                   | 1.5E+02 TCID <sub>50</sub> /mL                   |
| Dengue Virus 3                        | H87                                        | 1.3E+02                   | 3.7E+00                                          |
| Dengue Virus 4                        | H241                                       | 6.4E+01                   | 1.8E+02                                          |
| <i>Ebolavirus</i> spp.                | Bundibugyo: 200706291 Uganda (Inactivated) | 7.0E+04                   | N/A <sup>1</sup>                                 |
|                                       | Bundibugyo/Uganda (811250) (Live)          | 7.0E+02 <sup>2</sup>      | 5.6E+00 PFU/mL                                   |
|                                       | Tai Forest: Cote d'Ivoire (inactivated)    | 8.3E+03                   | N/A <sup>1</sup>                                 |
|                                       | Tai Forest: Cote d'Ivoire (live)           | 1.8E+02 <sup>2</sup>      | N/A <sup>1</sup>                                 |
|                                       | Reston:119810 RIID (Inactivated)           | 2.7E+04                   | N/A <sup>1</sup>                                 |
|                                       | Reston/ H-28 (Live)                        | 2.7E+03 <sup>2</sup>      | 4.7E+01 PFU/mL                                   |
|                                       | Sudan: Boniface (Inactivated)              | 1.1E+04                   | N/A <sup>1</sup>                                 |
|                                       | Sudan: Boniface (Live)                     | 1.1E+02 <sup>2</sup>      | 5.3E+00 PFU/mL                                   |

| Global Fever Panel Test Result                    | Species/Strain Tested                             | LoD                       |                                                  |
|---------------------------------------------------|---------------------------------------------------|---------------------------|--------------------------------------------------|
|                                                   |                                                   | Based on qPCR (copies/mL) | Based on Provided Stock Concentration (units/mL) |
|                                                   | Zaire: Gueckedou/Guinea C07 (Inactivated)         | 1.0E+02                   | 1.5E+02 PFU/mL                                   |
|                                                   | Zaire/ Makona (Live)                              | 1.1E+03 <sup>2</sup>      | 1.6E+01 PFU/mL                                   |
| Lassa virus                                       | Josiah (Inactivated)                              | 5.6E+04                   | N/A <sup>1</sup>                                 |
|                                                   | Josiah (Live)                                     | 5.6E+03 <sup>2</sup>      | 2.6E+01 PFU/mL                                   |
| Marburgvirus                                      | Marburg virus: Musoke (Inactivated)               | 5.0E+02                   | N/A <sup>1</sup>                                 |
|                                                   | Marburg virus Ci67 (Live)                         | 5.0E+02 <sup>2</sup>      | 1.5E+03 PFU/mL                                   |
|                                                   | Ravn virus: Kenya Ravn (Inactivated)              | 2.6E+02                   | N/A <sup>1</sup>                                 |
|                                                   | Ravn virus (Live)                                 | 2.6E+02 <sup>2</sup>      | 4.7E+01 PFU/mL                                   |
| West Nile Virus                                   | NY 2001-6263 (Lineage 1) (Inactivated)            | 1.1E+03                   | 2.7E+01                                          |
|                                                   | B-956 Uganda (Lineage 2) (Inactivated)            | 2.3E+04                   | 6.2E+00                                          |
|                                                   | Bz NY99 Lineage 1 (Live)                          | 1.6E+02 <sup>2</sup>      | N/A <sup>1</sup>                                 |
| Yellow Fever Virus                                | Strain 17D (Live attenuated)                      | 1.2E+02                   | 1.2E+01 TCID <sub>50</sub> /mL                   |
|                                                   | Asibi (Live)                                      | 1.2E+01 <sup>2</sup>      | 9.2E+00 PFU/mL                                   |
| PROTOZOA                                          |                                                   |                           |                                                  |
| <i>Leishmania</i> spp.                            | <i>L. donovani</i> : 9515                         | 1.0E+01                   | 2.2E+01 cells/mL                                 |
| <i>Plasmodium</i> spp.                            | <i>P. falciparum</i> IPC4884 Pursat Cambodia 2011 | 1.8E+02                   | N/A <sup>1</sup>                                 |
|                                                   | <i>P. knowlesi</i> H Strain                       | 2.4E+01                   | 20 pg/mL                                         |
|                                                   | <i>P. malariae</i> clinical specimen              | 1.9E+02                   | 2.3E-01 cells/mL                                 |
|                                                   | <i>P. ovale</i> Wallikeri                         | 2.4E+02                   | N/A <sup>1</sup>                                 |
|                                                   | <i>P. vivax</i> Chesson                           | 1.5E+02                   | N/A <sup>1</sup>                                 |
| <i>Plasmodium falciparum</i>                      | IPC4884 Pursat Cambodia 2011                      | 1.8E+02                   | N/A <sup>1</sup>                                 |
| <i>Plasmodium vivax</i> / <i>Plasmodium ovale</i> | <i>P. ovale</i> Wallikeri                         | 2.4E+02                   | N/A <sup>1</sup>                                 |
|                                                   | <i>P. vivax</i> Chesson                           | 1.5E+02                   | N/A <sup>1</sup>                                 |

<sup>1</sup> Quantification of the organism stock material was not available.

<sup>2</sup>LoD for these live strains was only estimated

## 7. Assay Cut-Off:

The BioFire Global Fever Special Pathogens Panel Melt Detector software determines whether a BioFire Global Fever Special Pathogens Panel assay result is positive or negative using a predefined algorithm that includes T<sub>m</sub> values, fluorescence values, and analysis of melting curves.

Initial melt ranges for each analyte-specific BioFire Global Fever Special Pathogens Panel assay were determined based on a combination of mathematical modeling using known sequence variations of different strains/isolates/variants of targeted organisms as well as data from testing of clinical specimens and known isolates.

After completion of the analytical and clinical studies on the BioFire Global Fever Special Pathogens Panel, a final validation of the melt ranges was performed and included review of data from the Inclusivity study and clinical studies. The observed sensitivity and specificity rates for the individual melt curves and assay calls as compared to expert annotation was greater than 99.4% and 100% respectively. The sensitivity, specificity, and accuracy for the validation data were determined to be well above the acceptance criteria.

## B Comparison Studies:

1. Method Comparison with Predicate Device:  
Not applicable.
2. Matrix Comparison:

Archived clinical serum and plasma samples were utilized to support performance of the Global Fever Special Pathogens Panel for detection of West Nile Virus. To establish that no performance difference exists between serum or plasma and the claimed whole blood specimen matrix for this target pathogen, a matrix equivalency study was performed.

Briefly, whole blood was collected in K-EDTA tubes and in serum separator tubes (SST) for serum separation. Blood from K-EDTA tubes was spiked with inactivated virus and divided to produce a whole blood sample intended for direct testing, with the remainder being processed to obtain virus-spiked plasma samples. Blood in SST tubes was processed to obtain serum which was spiked with the same concentration of inactivated WNV.

Plasma and serum samples are not intended to be used with the Global Fever Special Pathogens Panel. Results from this study are summarized in the table below.

**Table 21. Matrix Equivalency Study for Archived WNV Samples**

| Analyte                         | Concentration Tested<br>( $\times$ LoD)                        | Sample Matrix | Detection (%) | Cp Values |     |
|---------------------------------|----------------------------------------------------------------|---------------|---------------|-----------|-----|
|                                 |                                                                |               |               | Mean      | SD  |
| WNVLineage<br>1<br>NY 2001-6263 | <b>5.5E+03<br/>Copies/mL<br/>(5<math>\times</math> LoD)</b>    | Whole Blood   | 10/10 (100%)  | 16.7      | 0.7 |
|                                 |                                                                | Plasma        | 10/10 (100%)  | 15.1      | 0.8 |
|                                 |                                                                | Serum         | 10/10 (100%)  | 16.2      | 1.8 |
|                                 | <b>2.2E+03<br/>Copies/mL<br/>(2<math>\times</math> LoD)</b>    | Whole Blood   | 30/30 (100%)  | 18.4      | 1.0 |
|                                 |                                                                | Plasma        | 30/30 (100%)  | 16.7      | 0.6 |
|                                 |                                                                | Serum         | 30/30 (100%)  | 18.1      | 1.1 |
|                                 | <b>1.1E+02<br/>Copies/mL<br/>(0.1<math>\times</math> LoD)</b>  | Whole Blood   | 10/10 (100%)  | 22.1      | 1.1 |
|                                 |                                                                | Plasma        | 10/10 (100%)  | 21.2      | 0.4 |
|                                 |                                                                | Serum         | 9/10 (90%)    | 22.1      | 0.7 |
|                                 | <b>1.1E+01<br/>Copies/mL<br/>(0.01<math>\times</math> LoD)</b> | Whole Blood   | 1/10 (10%)    | 23.5      | -   |
|                                 |                                                                | Plasma        | 8/10 (80%)    | 24.7      | 1.9 |
|                                 |                                                                | Serum         | 7/10 (70%)    | 23.4      | 0.9 |
|                                 | <b>Negative</b>                                                | Whole Blood   | 0/10 (0%)     | -         | -   |
|                                 |                                                                | Plasma        | 0/10 (0%)     | -         | -   |
|                                 |                                                                | Serum         | 0/10 (0%)     | -         | -   |

These data support the sponsor's reliance on archived plasma and serum samples in their validation studies.

## **C Clinical Studies:**

### **1. Clinical Sensitivity:**

#### **Prospective Clinical Study**

The clinical performance of the Global Fever Special Pathogens Panel was established during a multi-center study conducted at eleven geographically distinct study sites, including two in the United States, from March 2018 – March 2021.

Prospective whole blood specimens were collected from subjects meeting the following eligibility criteria:

##### **Inclusion criteria**

- Subject has a recorded or self-reported fever within the past two days
- Subject provides informed consent prior to enrollment and specimen collection
- Subject has not participated in the study within the last 30 days

##### **Exclusion criteria**

- Subject does not have or did not self-report having a fever within the past two days
- Subject was unable to provide informed consent
- Subject has participated in the study within the last 30 days.

As recorded in site-specific study protocols, some sites applied additional eligibility criteria such as age limits, citizenship within the host country, and/or suspicion in the opinion of the physician that the subject's fever could be due to a pathogen on the BioFire Global Fever Special Pathogens Panel.

A total of 2,249 collected specimens were generated across all study sites and 110 subjects or their specimens were excluded from the final data analysis. The most common reason for exclusion was difficulty drawing blood (51/110). Thirty-eight (38/110) were excluded due to errors by study personnel. Fifteen specimens (15/110) were excluded due to inability to obtain a BioFire Global Fever Special Pathogens Panel or comparator result and six (6/110) were excluded because of the subject withdrawing before the specimen was collected. In total, 2,249 subjects were enrolled, 2,191 specimens were collected, 2,168 eligible specimens were tested on the Global Fever Panel, and 2,139 specimens were included in the final analyses.

Table 22 below shows the participating study sites and the number of prospective specimens enrolled at each study site.

**Table 22. Prospectively Collected Samples Across Sites in the BioFire Global Fever Special Pathogens Panel Clinical Study**

| Study Site (Location)   | Study Site # | Enrolled Population           | Total Subjects Enrolled | Total Valid WB Specimens Enrolled (excluded) | Study Started – Completed (2016) |
|-------------------------|--------------|-------------------------------|-------------------------|----------------------------------------------|----------------------------------|
| USA                     | Site 7       | Emergency Department (ED)     | 200                     | 179 (21)                                     | August 2018- November 2018       |
|                         | Site 14      | Inpatient and ED              | 90                      | 79 (7)                                       | August 2019- November 2020       |
| Africa                  | Site 1       | Inpatient                     | 145                     | 134 (9)                                      | March 2018- May 2019             |
|                         | Site 2       | Inpatient and Outpatient      | 200                     | 189 (9)                                      | June 2019- January 2020          |
|                         | Site 4       |                               | 113                     | 113 (0)                                      | July 2020- March 2021            |
|                         | Site 5       | Inpatient and Outpatient      | 203                     | 199 (4)                                      | January 2019- June 2019          |
|                         | Site 11      | Inpatient and Outpatient      | 163                     | 158 (4)                                      | July 2019- September 2019        |
| Southeast Asia          | Site 8       | Inpatient and Outpatient      | 280                     | 249 (31)                                     | November 2018 – September 2019   |
|                         | Site 9       | Outpatient                    | 415                     | 406 (9)                                      | February 2019 – September 2019   |
| Central & South America | Site 12      | Outpatient                    | 300                     | 297 (1)                                      | November 2018 – July 2019        |
|                         | Site 13      | Inpatient, Outpatient, and ED | 140                     | 136 (3)                                      | August 2019 – November 2020      |
| Total                   |              |                               | 2249                    | 2139 (110)                                   | March 2018- March 2021           |

Table 23 below shows breakdown of gender and age in clinical study.

**Table 23. Overall and Per Site Demographic Analysis for the Global Fever Panel Clinical Study**

|       |               | Overall      | Site #1    | Site #2     | Site #4    | Site #5     | Site #7                | Site #8     | Site #9                  | Site 11    | Site #12    | Site #13   | Site #14   |
|-------|---------------|--------------|------------|-------------|------------|-------------|------------------------|-------------|--------------------------|------------|-------------|------------|------------|
| Sex   | Male          | 1044 (48.8%) | 58 (43.3%) | 115 (60.8%) | 31 (27.4%) | 125 (62.8%) | 113 (63.1%)            | 107 (43.0%) | 206 (50.7%)              | 87 (55.1%) | 132 (44.4%) | 80 (58.8%) | 41 (51.9%) |
|       | Female        | 1095 (51.2%) | 76 (56.7%) | 74 (39.2%)  | 82 (72.6%) | 74 (37.2%)  | 66 (36.9%)             | 142 (57.0%) | 200 (49.3%)              | 71 (44.9%) | 165 (55.6%) | 56 (41.2%) | 38 (48.1%) |
| Age   | < 5 years     | 178 (8.3%)   | 44 (32.8%) | 35 (18.5%)  | 0 (0%)     | 0 (0%)      | 0 (0%) <sup>a</sup>    | 25 (10.0%)  | 0 (0%) <sup>b</sup>      | 66 (41.8%) | 3 (1.0%)    | 5 (3.7%)   | 0 (0%)     |
|       | 5 – 21 years  | 822 (38.4%)  | 21 (15.7%) | 78 (41.3%)  | 14 (12.4%) | 128 (64.3%) | 14 (7.8%) <sup>a</sup> | 127 (51%)   | 204 (50.2%) <sup>b</sup> | 70 (44.3%) | 102 (34.3%) | 61 (44.9%) | 3 (3.8%)   |
|       | 22 – 50 years | 779 (36.4%)  | 42 (31.3%) | 46 (24.3%)  | 62 (54.9%) | 59 (29.6%)  | 106 (59.2%)            | 63 (25.3%)  | 141 (34.7%)              | 21 (13.3%) | 146 (49.2%) | 61 (44.9%) | 32 (40.5%) |
|       | 50+ years     | 360 (16.8%)  | 27 (20.1%) | 30 (15.9%)  | 37 (32.7%) | 12 (6%)     | 59 (33.0%)             | 34 (13.7%)  | 61 (15.0%)               | 1 (0.6%)   | 46 (15.5%)  | 9 (6.6%)   | 44 (55.7%) |
| Total |               | 2139         | 134        | 189         | 113        | 189         | 179                    | 249         | 406                      | 158        | 297         | 136        | 79         |

<sup>a</sup> Site was not enrolling subjects <18 years old

<sup>b</sup> Site was not enrolling subjects <7 years old

In the laboratory, specimens were thoroughly mixed by vortexing or inverting and pipetted into a 700-μL aliquot for FilmArray testing, a 1300-μL aliquot for nucleic acid extraction, and the remaining volume (typically ≥500 μL) into an aliquot for archiving. Extracted

nucleic acid was stored at  $\leq 70^{\circ}\text{C}$  and shipped on dry ice to BioFire Defense for molecular comparator testing.

0.2% (5/2168) of eligible specimens were ultimately excluded due to inability to obtain a Global Fever Special Pathogens Panel test result. The overall success rate for initial specimen tests on the Global Fever Special Pathogens Panel was 98.5% (2136/2168); Seven tests did not complete (four due to loss of power, two due to instrument errors, and one due to a software error) and 25 had pouch internal control failures. Of the 32 unsuccessful initial tests, all were retested once, and valid results were produced for 27 of the 32 retested specimens.

The incomplete runs and instrument errors accounted for 0.3% (7/2168) of initial runs, resulting in an instrument success rate of 99.7% (2161/2168). Of the seven unsuccessful initial tests due to instrument performance, all were retested once, and three produced valid results. The two specimens that failed after retesting were excluded from further analysis.

Of the 2161 initial tests that completed, 1.2% (25/2161) did not produce valid internal controls, resulting in a pouch control success rate of 98.8% (2136/2161). Of the 25 tests with internal control failures, all were retested once, and 22 produced valid control results. Internal controls failed a second time for three specimens upon retesting, so these three specimens were excluded from further analysis.

All specimens were evaluated with the BioFire Global Fever Special Pathogens Panel at the clinical study sites. Frozen nucleic acid extracts were sent to BioFire Defense for comparator testing with well-validated nested PCR assays followed by bi-directional Sanger sequencing. Two comparator assays were utilized for each assay target with positive result from both assays considered positive. If the results of the two comparator assays for a particular analyte disagreed, the samples were subjected to repeat comparator testing with samples determined as positive if at least 2/3 replicates were positive for a single comparator assay.

PPA for each analyte was calculated as  $100\% \times (\text{TP}/(\text{TP} + \text{FN}))$ . True positive (TP) indicates that both the BioFire Global Fever Special Pathogens Panel and the comparator method had a positive result for this specific analyte, and false negative (FN) indicates that the BioFire Global Fever Special Pathogens Panel result was negative while the comparator result was positive. NPA was calculated as  $100\% \times (\text{TN}/(\text{TN} + \text{FP}))$ . True negative (TN) indicates that both the BioFire Global Fever Special Pathogens Panel and the comparator method had negative results and a false positive (FP) indicates that the BioFire Global Fever Special Pathogens Panel result was positive, but the comparator result was negative. The exact binomial two-sided 95% confidence interval was calculated. Samples for which false positive and/or false negative results (i.e., discrepant results) were obtained when comparing the BioFire Global Fever Special Pathogens Panel results to the comparator method results were further investigated. The discrepancy investigations were typically performed as follows: 1) All FP and FN were examined to determine whether additional testing on Global Fever Panel or with comparator assays could detect the analyte, but initial testing reported as not detected or negative because analyte was near or below the detection threshold; 2) FP and FN were evaluated by at least one additional PCR test that used different primers than the Global Fever Special Pathogens Panel assay or the comparator assays; 3) When possible, unresolved discrepancies were evaluated with additional PCR testing that could be verified by sequence analysis. Discrepancy results are footnoted below in Table 24.

**Table 24. FilmArray Global Fever Special Pathogens Panel Clinical Performance Summary**

| Analyte                                              |         | Positive Percent Agreement |       |            | Negative Percent Agreement |       |            |
|------------------------------------------------------|---------|----------------------------|-------|------------|----------------------------|-------|------------|
|                                                      |         | TP/(TP + FN)               | %     | 95%CI      | TN/(TN + FP)               | %     | 95% CI     |
| <b>VIRUSES</b>                                       |         |                            |       |            |                            |       |            |
| Chikungunya virus <sup>a</sup>                       | Fresh   | 25/25                      | 100%  | 86.7-100%  | 1442/1444                  | 99.9% | 99.5-100%  |
|                                                      | Frozen  | 0/0                        | -     | -          | 406/406                    | 100%  | 99.1-100%  |
|                                                      | Overall | 25/25                      | 100%  | 86.7-100%  | 1848/1850                  | 99.9% | 99.6-100%  |
| Crimean-Congo hemorrhagic fever virus                | Fresh   | 0/0                        | 0%    | -          | 1720/1720                  | 100%  | 99.8-100%  |
|                                                      | Frozen  | 1/1                        | 100%  | 20.7-100%  | 418/418                    | 100%  | 99.8-100%  |
|                                                      | Overall | 1/1                        | 100%  | 20.7-100%  | 2138/2138                  | 100%  | 99.8-100%  |
| Dengue Virus (serotypes 1, 2, 3, and 4) <sup>b</sup> | Fresh   | 249/263                    | 94.7% | 91.3-96.8% | 1206/1206                  | 100%  | 99.7-100%  |
|                                                      | Frozen  | 17/20                      | 85.0% | 64.0-94.8  | 386/386                    | 100%  | 99-100%    |
|                                                      | Overall | 266/283                    | 94.0% | 90.6-96.2% | 1592/1592                  | 100%  | 99.8-100%  |
| Ebola virus                                          | Fresh   | 0/0                        | -     | -          | 1720/1720                  | 100%  | 99.8-100%  |
|                                                      | Frozen  | 0/0                        | -     | -          | 419/419                    | 100%  | 99.1-100%  |
|                                                      | Overall | 0/0                        | -     | -          | 2139/2139                  | 100%  | 99.8-100%  |
| Lassa virus                                          | Fresh   | 0/0                        | -     | -          | 1720/1720                  | 100%  | 99.8-100%  |
|                                                      | Frozen  | 0/0                        | -     | -          | 419/419                    | 100%  | 99.1-100%  |
|                                                      | Overall | 0/0                        | -     | -          | 2139/2139                  | 100%  | 99.8-100%  |
| Marburg virus                                        | Fresh   | 0/0                        | -     | -          | 1720/1720                  | 100%  | 99.8-100%  |
|                                                      | Frozen  | 0/0                        | -     | -          | 419/419                    | 100%  | 99.1-100%  |
|                                                      | Overall | 0/0                        | -     | -          | 2139/2139                  | 100%  | 99.8-100%  |
| West Nile Virus                                      | Fresh   | 1/1                        | 100%  | 20.7-100%  | 1719/1719                  | 100%  | 99.8-100%  |
|                                                      | Frozen  | 0/0                        | -     | -          | 419/419                    | 100%  | 99.1-100%  |
|                                                      | Overall | 1/1                        | 100%  | 20.7-100%  | 2138/2138                  | 100%  | 99.8-100%  |
| Yellow Fever Virus                                   | Fresh   | 0/0                        | -     | -          | 1720/1720                  | 100%  | 99.8-100%  |
|                                                      | Frozen  | 0/0                        | -     | -          | 419/419                    | 100%  | 99.1-100%  |
|                                                      | Overall | 0/0                        | -     | -          | 2139/2139                  | 100%  | 99.8-100%  |
| <b>BACTERIA</b>                                      |         |                            |       |            |                            |       |            |
| <i>Bacillus anthracis</i>                            | Fresh   | 0/0                        | -     | -          | 1720/1720                  | 100%  | 99.8-100%  |
|                                                      | Frozen  | 0/0                        | -     | -          | 419/419                    | 100%  | 99.1-100%  |
|                                                      | Overall | 0/0                        | -     | -          | 2139/2139                  | 100%  | 99.8-100%  |
| <i>Francisella tularensis</i>                        | Fresh   | 0/0                        | -     | -          | 1720/1720                  | 100%  | 99.8-100%  |
|                                                      | Frozen  | 0/0                        | -     | -          | 419/419                    | 100%  | 99.1-100%  |
|                                                      | Overall | 0/0                        | -     | -          | 2139/2139                  | 100%  | 99.8-100%  |
| <i>Leptospira</i> spp. <sup>c</sup>                  | Fresh   | 9/9                        | 100%  | 70.1-100%  | 1456/1460                  | 99.7% | 99.3-99.9% |
|                                                      | Frozen  | 6/7                        | 85.7% | 48.7-97.4% | 399/399                    | 100%  | 99-100%    |
|                                                      | Overall | 15/16                      | 93.8% | 71.7-98.9% | 1855/1859                  | 99.8% | 99.4-99.9% |
| <i>Yersinia Pestis</i>                               | Fresh   | 0/0                        | -     | -          | 1720/1720                  | 100%  | 99.8-100%  |
|                                                      | Frozen  | 0/0                        | -     | -          | 419/419                    | 100%  | 99.1-100%  |
|                                                      | Overall | 0/0                        | -     | -          | 2139/2139                  | 100%  | 99.8-100%  |
| <b>PROTOZOA</b>                                      |         |                            |       |            |                            |       |            |
| <i>Leishmania</i>                                    | Fresh   | 10/10                      | 100%  | 72.2-100%  | 1710/1710                  | 100%  | 99.8-100%  |
|                                                      | Frozen  | 0/0                        | -     | -          | 419/419                    | 100%  | 99.1-100%  |
|                                                      | Overall | 10/10                      | 100%  | 72.2-100%  | 2129/2129                  | 100%  | 99.8-100%  |

| Analyte                                    |         | Positive Percent Agreement |       |             | Negative Percent Agreement |        |            |
|--------------------------------------------|---------|----------------------------|-------|-------------|----------------------------|--------|------------|
|                                            |         | TP/(TP + FN)               | %     | 95%CI       | TN/(TN + FP)               | %      | 95% CI     |
| <i>Plasmodium</i> spp. <sup>d,e</sup>      | Fresh   | 206/209                    | 98.6% | 95.9-99.5%  | 1252/1261                  | 99.3%  | 98.7-99.7% |
|                                            | Frozen  | 132/134                    | 98.5% | 994.7-99.6% | 267/271                    | 98.5%  | 96.3-99.4% |
|                                            | Overall | 338/343                    | 98.5% | 96.6-99.4%  | 1519/1532                  | 99.2%  | 98.6-99.5% |
| <i>Plasmodium falciparum</i> <sup>f</sup>  | Fresh   | 148/158                    | 93.7% | 88.7-96.5%  | 1309/1311                  | 99.8%  | 99.4-100%  |
|                                            | Frozen  | 82/90                      | 91.1% | 83.4-95.4%  | 315/316                    | 99.7%  | 98.2-99.9% |
|                                            | Overall | 230/248                    | 92.7% | 88.8-95.4%  | 1624/1627                  | 99.8%  | 99.5-99.9% |
| <i>Plasmodium vivax/ovale</i> <sup>g</sup> | Fresh   | 64/69                      | 92.8  | 84.1-96.9%  | 1400/1400                  | 100.0% | 99.7-100%  |
|                                            | Frozen  | 51/55                      | 92.7% | 92.7-97.1%  | 351/351                    | 100.0% | 98.9-100%  |
|                                            | Overall | 115/124                    | 92.7% | 86.8-96.1%  | 1751/1751                  | 100.0% | 99.8-100%  |

<sup>a</sup>2/2 FP Chikungunya virus specimens were positive by additional PCR

<sup>b</sup>Evidence of Dengue virus was found in 15/17 FN specimens; five were positive upon retesting with Global Fever Panel and additional PCR, two were positive upon Retest with Global Fever Special Pathogens Panel, and eight were detected only by additional PCR.

<sup>c</sup>Evidence of *Leptospira* spp. was found in 1/1 FN by GF Special Pathogens panel retest and by additional PCR and in 3/4 FP specimens by additional PCR.

<sup>d</sup>Four (4/5) *Plasmodium* spp. FN specimens were *P. falciparum* FN and 1/5 was *P. vivax/ovale*. Three (3/13) *Plasmodium* spp. FP specimens were also *P. falciparum* FP

<sup>e</sup>*Plasmodium* spp. were detected in 2/5 FN Specimens: one was positive upon GF Special Pathogens Panel retest and additional PCR, one was positive only upon GF Special Pathogens Panel retest. *Plasmodium* spp. were also detected in 11/13 FP specimens by additional PCR.

<sup>f</sup>Evidence of *Plasmodium* spp. was found in 13/18 FN specimens: three upon GF Panel retest and by additional PCR, nine were only positive by additional PCR. *P. falciparum* was detected in 2/3 FP specimens by additional PCR.

<sup>g</sup>*P. vivax/ovale* was detected in 7/9 FN specimens: two of which were positive both upon GF Panel retest and by additional PCR, two were positive only by GF Panel retest, and three were positive only by additional PCR.

Thirty-one specimens had a positive *Plasmodium* spp. Detected result with a concomitant Not Detected result for either *P. falciparum* or *P. vivax/ovale*. For 19 of these specimens, Cp value of the *Plasmodium* spp. assay was near or later than the LoD range and/or comparator testing identified *P. falciparum* or *P. vivax/ovale* infection. A further 8/31 of these specimens had robust *Plasmodium* spp. amplification but no further evidence of *P. falciparum* or *P. vivax/ovale* infection. These cases likely reflect infection with a different malaria species (*P. knowlesi* or *P. malariae*) not differentiated by the Global Fever Special Pathogens Panel malaria assays.

When possible, performance of malaria testing as part of Standard of Care was compared to the Global Fever Special Pathogens Panel result. Because most sites did not attempt to speciate malaria, only the *Plasmodium* spp. assay result was used in this analysis. Each clinical site followed its own standard procedure for malaria testing with significant differences between sites. Data is listed in Table 25 below as Site-specific PPA and NPA with site-specific microscopic results.

**Table 25. Performance Comparison Between Global Fever Special Pathogens Panel and Site-Specific Malaria Testing.**

| Site (Method)               | Site PPA                |            | Site NPA                |            |
|-----------------------------|-------------------------|------------|-------------------------|------------|
|                             | Site Positive Agreement | 95% CI     | Site Negative Agreement | 95% CI     |
| 02 (Thick Smear and/or RDT) | 91.8% (45/49)           | 80.8-96.8% | 52.8% (66/125)          | 44.1-61.3% |
| 05a (Thick Smear)           | 100% (62/62)            | 94.2-100%  | 43.3% (13/30)           | 27.4-60.8% |
| 05b (Thick/Thin Smear)      | 90.0% (27/30)           | 74.4-96.5% | 60.3% (38/63)           | 48.0-71.5% |
| 09b (Thin Smear)            | 100% (3/3)              | 43.9-100%  | 100% (8/8)              | 67.6-100%  |
| 11c (Thick Smear)           | 100% (3/3)              | 43.9-100%  | 0/0                     | -          |

|                   |                        |                   |                        |                   |
|-------------------|------------------------|-------------------|------------------------|-------------------|
| 11d (Thick Smear) | 0/0                    | -                 | 100% (1/1)             | 20.7-100%         |
| 11e (Thin Smear)  | 100% (3/3)             | 43.9-100%         | 0/0                    | -                 |
| 11f (Thin Smear)  | 100% (11/11)           | 74.1-100%         | 0/0                    | -                 |
| 12a (Thick Smear) | 100% (53/53)           | 93.2-100%         | 98.7% (74/75)          | 92.8-99.8%        |
| 12c (Thick Smear) | 100% (17/17)           | 81.6-100%         | 92.8% (90/97)          | 85.8-96.5%        |
| 12d (Thick Smear) | 100% (5/5)             | 56.6-100%         | 97.6% (41/42)          | 87.7-99.6%        |
| 13 (Thick Smear)  | 0/0                    | -                 | 100% (1/1)             | 20.7-100%         |
| <b>Overall</b>    | <b>97.0% (229/236)</b> | <b>94.0-98.6%</b> | <b>75.1% (332/442)</b> | <b>70.9-78.9%</b> |

Overall, the Global Fever Special Pathogens Panel showed strong correlation with site-specific methods when the site was able to detect malaria. Importantly, for 5 of the false negative results, the comparator method agreed with Global Fever Special Pathogens Panel. The Global Fever Special Pathogens Panel was also able to detect *Plasmodium* in many specimens that were determined to be negative by site-specific methods (110/442). For 71/81 of these Detections, the comparator method agreed with the Global Fever Panel result. While it cannot be ruled out that the Global Fever Panel detected residual nucleic acid following a cleared infection, these results could also reflect that the Global Fever Panel is more sensitive than the other methods utilized.

The Global Fever Special Pathogens Panel reported a total of 31 specimens with discernible multiple analyte detections (1.4% of all specimens, 31/2139; 4.2% of positive specimens, 31/730). The majority of co-detections (29/31; 93.5%) contained *Plasmodium*. No co-detections contained more than two discernible analytes (Table 89). The most frequently occurring combination of analytes was *P. falciparum* with *P. vivax/ovale* (3.4% of all positive specimens; 25/730).

**Table 26. Global Fever Special Pathogens Panel Clinical Study Specimen Co-Detections**

| Distinct Co-detection Combinations  |                                     |                       | Total Co-detections |
|-------------------------------------|-------------------------------------|-----------------------|---------------------|
| Analyte 1                           | Analyte 2                           | Analyte 3             |                     |
| Chikungunya virus <sup>a</sup>      | Dengue virus                        | -                     | 2                   |
| Dengue virus                        | <i>Plasmodium</i> spp.              | <i>P. falciparum</i>  | 1                   |
| Dengue virus                        | <i>Plasmodium</i> spp.              | <i>P. vivax/ovale</i> | 1                   |
| <i>Leptospira</i> spp. <sup>b</sup> | <i>Plasmodium</i> spp. <sup>b</sup> | -                     | 1                   |
| <i>Leptospira</i> spp.              | <i>Plasmodium</i> spp.              | <i>P. vivax/ovale</i> | 1                   |
| <i>Plasmodium</i> spp.              | <i>P. falciparum</i>                | <i>P. vivax/ovale</i> | 25                  |

<sup>a</sup>Chikungunya virus was detected by Global Fever Special Pathogens Panel but not by comparator testing in one of the two co-detections

<sup>b</sup>Comparator testing did not identify *Leptospira* spp or *Plasmodium* spp. in this specimen.

Certain analytes on the BioFire Global Fever Special Pathogens Panel were either not observed or were not encountered in large enough numbers to adequately demonstrate system performance during the prospective clinical evaluation. Therefore, archived specimens with known analyte content and/or archived specimens with a high likelihood of containing a given analyte were tested using the BioFire Global Fever Special Pathogens Panel to supplement the prospective clinical evaluation data. Wherever possible, archived WB specimens were tested. Where no WB specimens could be obtained blood plasma and blood serum were tested instead. Although plasma and serum are not the intended specimen type for the Global Fever Special Pathogens Panel, these blood components are expected to

provide similar results to WB specimens. Results from testing of archived specimens are summarized below.

**Table 27. BioFire Global Fever Special Pathogens Panel Archived Specimen Performance Summary**

| Analyte                               | Positive Percent Agreement |       |            | Negative Percent Agreement |       |            |
|---------------------------------------|----------------------------|-------|------------|----------------------------|-------|------------|
|                                       | TP/(TP + FN)               | %     | 95%CI      | TN/(TN + FP)               | %     | 95% CI     |
| <b>Viruses</b>                        |                            |       |            |                            |       |            |
| Crimean-Congo hemorrhagic fever virus | 0/0                        | -     | -          | 281/281                    | 100%  | 98.7-100%  |
| Ebola virus <sup>a</sup>              | 0/0                        | -     | -          | 279/279                    | 100%  | 98.6-100%  |
| Lassa virus <sup>a,b</sup>            | 10/12                      | 83.3% | 55.2-95.3% | 265/267                    | 99.2% | 97.3-100%  |
| Marburg virus <sup>a</sup>            | 0/0                        | -     | -          | 279/279                    | 100%  | 98.6-100%  |
| West Nile virus <sup>a,c,d,e</sup>    | 59/65                      | 90.8% | 81.3-95.7% | 345/347                    | 99.4% | 97.9-99.8% |
| Yellow Fever Virus <sup>a</sup>       | 0/0                        | -     | -          | 279/279                    | 100%  | 98.6-100%  |
| <b>Bacteria</b>                       |                            |       |            |                            |       |            |
| <i>Bacillus anthracis</i>             | 0/0                        | -     | -          | 281/281                    | 100%  | 98.7-100%  |
| <i>Francisella tularensis</i>         | 0/0                        | -     | -          | 281/281                    | 100%  | 98.7-100%  |
| <i>Yersinia pestis</i>                | 0/0                        | -     | -          | 281/281                    | 100%  | 98.7-100%  |
| <b>Protozoa</b>                       |                            |       |            |                            |       |            |
| <i>Leishmania spp.</i>                | 0/0                        | -     | -          | 283/283                    | 100%  | 98.7-100%  |

<sup>a</sup>Due to low specimen volume, comparator results were not obtained for two specimens

<sup>b</sup>Evidence of Lassa virus was found in 1/2 false negative specimens and 1/2 false positive specimens by additional PCR testing.

<sup>c</sup>Specimens tested at Site 03 were previously characterized and expected to be negative for all panel targets but positive for West Nile virus and were only evaluated by comparator methods for West Nile virus

<sup>d</sup>Archived specimens included blood serum and blood plasma

<sup>e</sup>Evidence of West Nile Virus was found in 4/4 false negative specimens; two specimens were positive upon retesting with the Global Fever Special Pathogens Panel and by additional PCR, and two were positive only by additional PCR testing. Evidence of West Nile Virus was detected in 1/2 false positive specimens by additional PCR testing.

Prospective sites were selected in an effort to collect specimens containing all pathogens on the Global Fever Special Pathogens Panel. Whenever possible, archived specimens containing analytes not observed in the prospective clinical evaluation were tested instead. For analytes for which no archived specimens were available, or for which there were an insufficient number of archived specimens, testing was performed using contrived specimens.

Contrived specimens were prepared using residual human WB specimens from patients with signs/symptoms of AFI. For each analyte, fifty replicates were contrived using quantified isolates at a range of concentrations relative to the LoD. If known, clinically relevant concentrations were used to adjust testing levels. The contrived specimens also served as a negative replicate for all other analytes. Specimens were prepared and randomized such that the analyte status of each contrived specimen was blinded to the users performing testing. The positive percent agreement (PPA) and negative percent agreement (NPA) were defined as agreement between the FilmArray Global Fever Special Pathogens Panel result and the known composition of the contrived specimen. A summary of the contrived specimen testing results are included in Table 28 below.

**Table 28. BioFire Global Fever Special Pathogens Panel Contrived Specimen Study Performance**

| Analyte                                            | Positive Percent Agreement |      |            | Negative Percent Agreement |      |           |
|----------------------------------------------------|----------------------------|------|------------|----------------------------|------|-----------|
|                                                    | TP/(TP + FN)               | %    | 95%CI      | TN/(TN + FP)               | %    | 95% CI    |
| <b>Viruses</b>                                     |                            |      |            |                            |      |           |
| Crimean-Congo hemorrhagic fever virus <sup>a</sup> | 98/100 <sup>b</sup>        | 98%  | 93.0-99.5% | 282/282                    | 100% | 98.7-100% |
| Ebola virus                                        | 50/50                      | 100% | 92.9-100%  | 332/332                    | 100% | 98.9-100% |
| Lassa virus                                        | 50/50                      | 100% | 92.9-100   | 332/332                    | 100% | 98.9-100% |
| Marburg virus <sup>a</sup>                         | 99/100 <sup>c</sup>        | 99%  | 94.6-99.8  | 282/282                    | 100% | 98.7-100% |
| West Nile virus                                    | 50/50                      | 100% | 92.9-100%  | 331/332 <sup>d</sup>       | 100% | 98.9-100% |
| Yellow Fever Virus                                 | 49/50 <sup>e</sup>         | 98%  | 89.5-99.7% | 332/332                    | 100% | 98.9-100% |
| <b>Bacteria</b>                                    |                            |      |            |                            |      |           |
| <i>Bacillus anthracis</i>                          | 50/50                      | 100% | 92.9-100%  | 332/332                    | 100% | 98.9-100% |
| <i>Francisella tularensis</i>                      | 50/50                      | 100% | 92.9-100%  | 332/332                    | 100% | 98.9-100% |
| <i>Yersinia pestis</i>                             | 50/50                      | 100% | 92.9-100%  | 332/332                    | 100% | 98.9-100% |
| <b>Protozoa</b>                                    |                            |      |            |                            |      |           |
| <i>Leishmania spp.</i>                             | 50/50                      | 100% | 92.9-100%  | 332/332                    | 100% | 98.9-100% |

<sup>a</sup>Additional testing was performed at concentrations that better represent the clinically relevant range

<sup>b</sup>Two false negative results were observed in specimens containing Crimean-Congo hemorrhagic fever virus at 2X LoD

<sup>c</sup>A single false negative result was observed in a specimen containing Marburgvirus spiked at 10X LoD

<sup>d</sup>Discrepancy testing identified near LoD levels of WNV in a single whole blood sample

<sup>e</sup>A single false negative result was observed in a specimen containing Yellow Fever virus spiked at 5X LoD

## 2. Clinical Specificity:

See clinical sensitivity section above.

## 3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not applicable.

## D Clinical Cut-Off:

Not applicable.

## E Expected Values/Reference Range:

The prevalence of individual analytes detected by the Global Fever Special Pathogens Panel as observed during the clinical study is described in Table 29 below. Analytes not identified in the table were not detected by the BioFire Global Fever Special Pathogens Panel in this study.



**Table 29. Prevalence of Detected Analytes Stratified by Region and Clinical Study Site**

| Analyte                                 | Overall<br>(N=2139) |       | USA                |      |                   |      | Africa             |       |                    |       |                    |       |                    |       | Southeast Asia     |       |                    |       | Europe             |      | Central & S. America |       |                 |       |
|-----------------------------------------|---------------------|-------|--------------------|------|-------------------|------|--------------------|-------|--------------------|-------|--------------------|-------|--------------------|-------|--------------------|-------|--------------------|-------|--------------------|------|----------------------|-------|-----------------|-------|
|                                         |                     |       | Site 07<br>(N=179) |      | Site 14<br>(N=79) |      | Site 01<br>(N=134) |       | Site 02<br>(N=189) |       | Site 05<br>(N=199) |       | Site 11<br>(N=158) |       | Site 08<br>(N=249) |       | Site 09<br>(N=406) |       | Site 04<br>(N=113) |      | Site 12<br>(N=297)   |       | Site 13 (N=136) |       |
|                                         | #                   | EV    | #                  | EV   | #                 | EV   | #                  | EV    | #                  | EV    | #                  | EV    | #                  | EV    | #                  | EV    | #                  | EV    | #                  | EV   | #                    | EV    | #               | EV    |
| Chikungunya virus                       | 27                  | 1.3%  | 0                  | 0.0% | 0                 | 0.0% | 0                  | 0.0%  | 0                  | 0.0%  | 0                  | 0.0%  | 0                  | 0.0%  | 0                  | 0.0%  | 27                 | 6.7%  | 0                  | 0.0% | 0                    | 0.0%  | 0               | 0.0%  |
| Crimean-Congo hemorrhagic fever virus   | 1                   | 0.05% | 0                  | 0.0% | 0                 | 0.0% | 0                  | 0.0%  | 0                  | 0.0%  | 1                  | 0.5%  | 0                  | 0.0%  | 0                  | 0.0%  | 0                  | 0.0%  | 0                  | 0.0% | 0                    | 0.0%  | 0               | 0.0%  |
| Dengue virus (Serotypes 1, 2, 3, and 4) | 266                 | 12.4% | 0                  | 0.0% | 0                 | 0.0% | 1                  | 0.7%  | 0                  | 0.0%  | 0                  | 0.0%  | 0                  | 0.0%  | 90                 | 36.1% | 54                 | 13.3% | 0                  | 0.0% | 20                   | 6.7%  | 101             | 74.3% |
| West Nile Virus                         | 1                   | 0.05% | 1                  | 0.6% | 0                 | 0.0% | 0                  | 0.0%  | 0                  | 0.0%  | 0                  | 0.0%  | 0                  | 0.0%  | 0                  | 0.0%  | 0                  | 0.0%  | 0                  | 0.0% | 0                    | 0.0%  | 0               | 0.0%  |
| <i>Leptospira</i> spp.                  | 19                  | 0.9%  | 1                  | 0.6% | 0                 | 0.0% | 0                  | 0.0%  | 1                  | 0.5%  | 0                  | 0.0%  | 0                  | 0.0%  | 4                  | 1.6%  | 4                  | 1.0%  | 0                  | 0.0% | 9                    | 3.0%  | 0               | 0.0%  |
| <i>Leishmania</i> spp.                  | 10                  | 0.5%  | 0                  | 0.0% | 0                 | 0.0% | 0                  | 0.0%  | 0                  | 0.0%  | 0                  | 0.0%  | 0                  | 0.0%  | 0                  | 0.0%  | 0                  | 0.0%  | 10                 | 8.8% | 0                    | 0.0%  | 0               | 0.0%  |
| <i>Plasmodium</i> spp.                  | 407                 | 19.0% | 0                  | 0.0% | 0                 | 0.0% | 16                 | 11.9% | 105                | 55.6% | 141                | 70.9% | 49                 | 31.0% | 7                  | 2.8%  | 4                  | 1.0%  | 1                  | 0.9% | 84                   | 28.3% | 0               | 0.0%  |
| <i>P. falciparum</i>                    | 283                 | 13.2% | 0                  | 0.0% | 0                 | 0.0% | 14                 | 10.4% | 94                 | 49.7% | 125                | 62.8% | 42                 | 26.6% | 3                  | 1.2%  | 0                  | 0.0%  | 0                  | 0.0% | 5                    | 1.7%  | 0               | 0.0%  |
| <i>P. vivax/ovale</i>                   | 118                 | 5.5%  | 0                  | 0.0% | 0                 | 0.0% | 3                  | 2.2%  | 3                  | 1.6%  | 12                 | 6.0%  | 12                 | 7.6%  | 4                  | 1.6%  | 4                  | 1.0%  | 0                  | 0.0% | 80                   | 26.9% | 0               | 0.0%  |

The BioFire Global Fever Special Pathogens Panel detected at least one analyte in 730 of the 2139 included specimens (34.1% positivity rate).



**Table 30. Expected Values Summary (Detections) as Determined in the BioFire Global Fever Special Pathogens Panel Clinical Study**

| FilmArray Result                 | Number Detected | % of Total<br>(% of Positives) |
|----------------------------------|-----------------|--------------------------------|
| Detected (at least one result)   | 730             | 34.1%<br>(100%)                |
| One analyte result               | 698             | 32.6%<br>(95.6%)               |
| Two analyte results <sup>a</sup> | 32              | 1.5%<br>(4.4%)                 |

<sup>a</sup>Data for discernible co-detections only. The *Plasmodium* spp. assay is not considered a unique Global Fever Panel detection when co-detected with a *Plasmodium* species-level assay. Specimens containing multiple species within a genus are not always discernible (e.g., a specimen containing *P. malariae* and *P. falciparum* would not produce a discernible co-detection.)

**Table 31. Expected Values Summary (Analytes) as Determined in the BioFire Global Fever Special Pathogens Panel Clinical Study**

| FilmArray Result                        | Number Detected | % of Positives |
|-----------------------------------------|-----------------|----------------|
| Chikungunya virus                       | 27              | 3.7%           |
| Crimean-Congo hemorrhagic fever virus   | 1               | 0.1%           |
| Dengue virus (serotypes 1, 2, 3, and 4) | 266             | 36.4%          |
| Ebola virus                             | 0               | 0%             |
| Lassa virus                             | 0               | 0%             |
| Marburg virus                           | 0               | 0%             |
| West Nile Virus                         | 1               | 0.1%           |
| Yellow Fever Virus                      | 0               | 0%             |
| <i>Bacillus anthracis</i>               | 0               | 0%             |
| <i>Francisella tularensis</i>           | 0               | 0%             |
| <i>Leptospira</i> spp.                  | 19              | 2.6%           |
| <i>Yersinia pestis</i>                  | 0               | 0%             |
| <i>Leishmania</i> spp.                  | 10              | 1.4%           |
| <i>Plasmodium</i> spp.                  | 407             | 55.8%          |
| <i>Plasmodium falciparum</i>            | 283             | 38.8%          |
| <i>Plasmodium vivax/ovale</i>           | 118             | 16.2%          |

The most prevalent analyte result was *Plasmodium* (407/730; 55.8%)—of which, 283/407 (69.5%) also had *Plasmodium falciparum* identified, 118/407 (29.0%) also had *Plasmodium vivax/ovale* identified, 31/407 (7.6%) had no species-level identification, and 25/407 (6.1%) had a combination of *P. falciparum* and *P. vivax/ovale*. The second most prevalent analyte was dengue virus (266/730, 36.4%). Chikungunya virus was detected in 3.7% (27/730) of specimens, *Leptospira* was detected in 2.6% (19/730), *Leishmania* was detected in 1.4% (10/730), and Crimean-Congo hemorrhagic fever virus, West Nile virus, Zika virus, and *Salmonella enterica* serovar Typhi were each detected in fewer than 1% of specimens (Table 3). All other analytes were not detected by the Global Fever Special Pathogens Panel in this study.

**F Other Supportive Instrument Performance Characteristics Data:**

Not applicable.

**VIII Proposed Labeling:**

The labeling supports the finding of substantial equivalence for this device.

**IX Conclusion:**

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.